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EVIDENCE OF VIRUS MUTATION IN THE COMMON MOSAIC OF TOBACCO [1]

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INTRODUCTION

In a short note (*12*) [2] the writer reported that Connecticut-Havana broadleaf tobacco [3] plants having common mosaic developed yellow spots on a few leaves, as previously illustrated (*15*), and as shown in figure 1, *B*. These yellow spots were observed regularly on plants in the more advanced stages of development, and very irregularly on plants when young, and then only when the young plants were growing at high temperatures.[4]

These yellow spots range in size from points just visible to areas approximating 1.5 cm^2 on the strain of tobacco used. On Turkish tobacco the spots are smaller and have been less consistent in occurrence. The large spots are less frequent, usually are very irregular in shape and not so yellow in color as the small spots, though small spots are not always completely devoid of chlorophyll. On this account one cannot be certain from mere observation that mosaic tissue is free of very small invisible centers of yellow-mosaic virus. Figure 2 illustrates a typical series of sizes and shapes of the yellow-mosaic spots.

It was found that these yellow spots contain virus which when introduced into young tobacco plants induced a large number of yellow areas on the young leaves, usually within 4 to 5 days at temperatures favorable for tobacco culture. A typical case is illustrated in figure 3, *A*. It was found that a very severe pure yellow mosaic sometimes could be obtained immediately in the inoculated plants when the virus was taken from the center of extremely yellow areas. When virus was taken from near the margins of the yellow areas, blends of yellow mosaic and green mosaic developed on many of the inoculated plants, and when virus was taken from the green areas common mosaic developed in the inoculated plants, but all of these plants ultimately developed a few small spots of yellow mosaic. The virus of common mosaic was completely removed from the yellow mosaic by successive subinoculations from the yellow-mosaic areas. Several leaves with yellow mosaic are illustrated in figure 1.

[1] Received for publication Aug. 26, 1935; issued February, 1936. These studies were started in 1926 at the University of Wisconsin and continued at the Arlington Experiment Farm, Rosslyn, Va.

[2] Reference is made by number (italic) to Literature Cited, p. 980.

[3] This variety, which was used throughout the present experiments, is now called Wisconsin-Havana Seed.

[4] It was stated (*12*) inadvertently that these yellow spots developed on young plants grown near 90° to 100° C. This temperature reading was taken from a Fahrenheit thermograph chart and through oversight was not converted to the centigrade scale. It should be read 32.2° to 37.7° C. These were midday temperatures during midsummer.

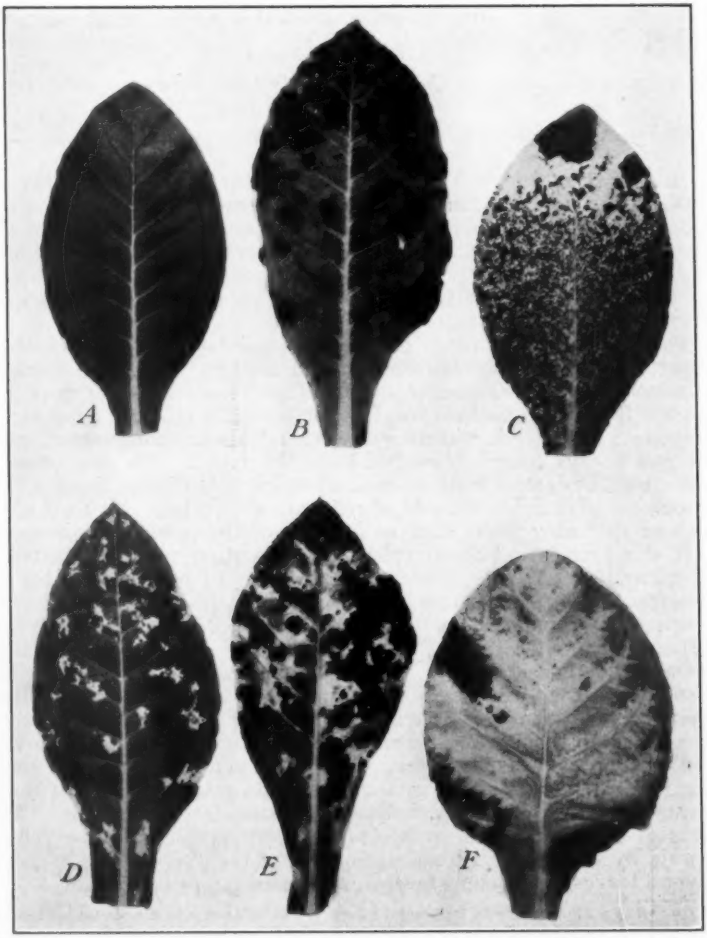

FIGURE 1.—Common mosaic and yellow mosaic on tobacco. *A*, Healthy leaf; *B*, leaf showing common mosaic and one yellow-mosaic spot; *C*, *D*, *E*, and *F*, several patterns of yellow mosaic produced by purified virus obtained from a yellow-mosaic spot such as is illustrated in *B*.

Many collections of mosaic have been studied, and as stated previously (*13*, *15*, *19*) all those having the appearance of common mosaic on tobacco have shown the presence of yellow-mosaic spots on one or more leaves on all of the inoculated plants. All attempts to obtain a common mosaic which does not develop yellow-mosaic spots failed.

In view of these facts it was pointed out (*15*) that the virus of common mosaic may mutate. In a later summary (*19*) the writer took the definite position that common green mosaic and the yellow mosaic associated with it do not represent an ordinary mixture of viruses. No statement was made at that time regarding mutation. However, common mosaic was referred to as a complex because all the evidence indicated that this disease on Wisconsin-Havana Seed tobacco when cultured under ordinary conditions represents a composite or aggregate of at least two types of mosaic—green mosaic which predominates and yellow mosaic which occurs in localized zones—and because of the never-failing recurrence of the yellow-

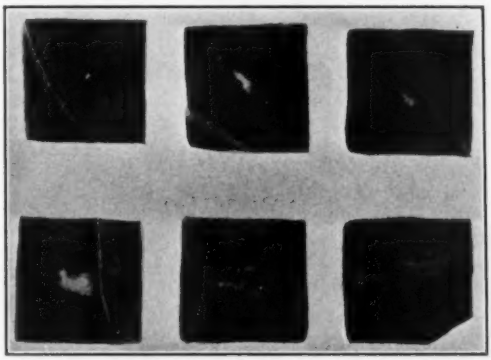

FIGURE 2.—Types of yellow-mosaic spots ranging from a small point to a large type in which some chlorophyll is present. Natural size.

mosaic phase with the green-mosaic phase of the disease in the writer's tests.[5]

Dufrenoy (*4*), working in France, and Jensen (*8*), in the United States, have verified the writer's findings that yellow mosaic is associated with the common mosaic of tobacco. Price (*23*) and Hoggan (*5*) have reported a similar phenomenon in cucumber mosaic. The writer (*16*, *18*) has pointed out that certain yellow and green mosaics of wheat are closely associated. Jensen (*8*) and Price (*23*) and Hoggan (*5*) consider that these associations in tobacco and cucumber mosaics do not constitute mixtures of an ordinary type, and that the viruses of yellow-mosaic spots arise in the plant sometime after inoculation.

The present paper deals with several lines of evidence which support the view that the viruses which cause the yellow-mosaic spots arise spontaneously as mutations of the virus of common mosaic, and are not the result of mixture or contamination of the ordinary

[5] Smith (*26*) reports a virus disease relationship in potato which he refers to as a complex, but this seems to represent an ordinary mechanical mixture of viruses. Thus, it is seen that the writer used the term complex in a different sense.

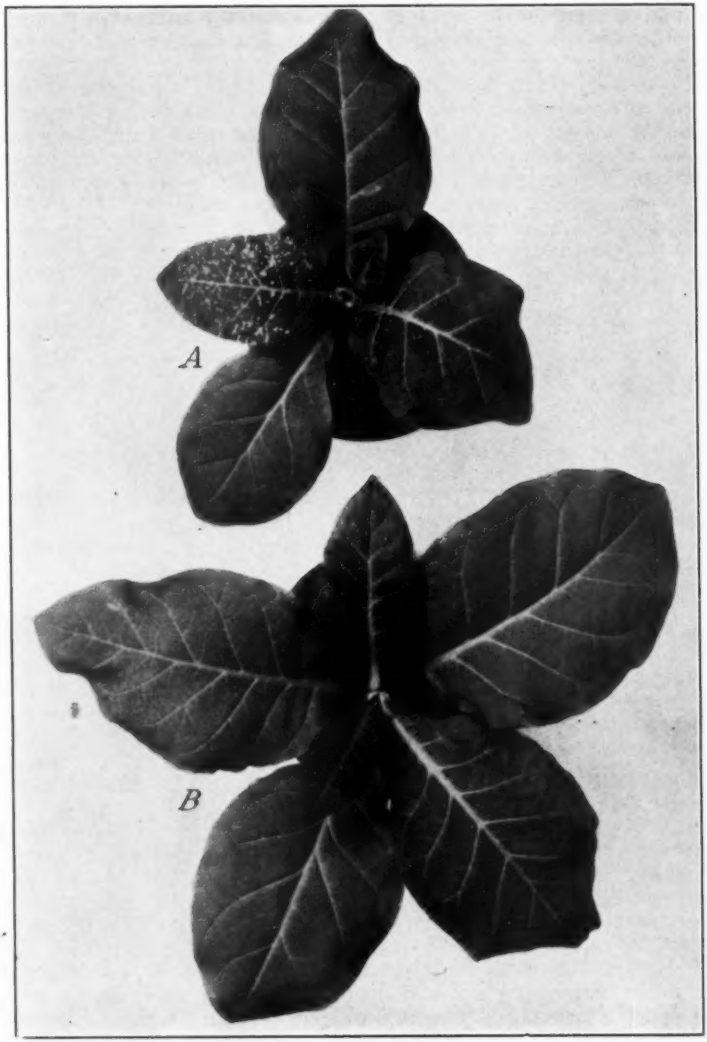

FIGURE 3.—*A*, first symptoms induced by virus from a yellow-mosaic spot; *B*, symptoms of common mosaic showing no yellow-mosaic spots induced by virus from the light- and dark-green areas of common mosaic. The new leaves shown in *A* and the subsequent leaves produced symptoms of common mosaic which were indistinguishable from the symptoms produced by plant *B*. Both plants produced a few yellow-mosaic spots before maturity.

sort running through all collections of common mosaic and through all of the many lots and fractions of inoculum used in the experimental work.

MATERIALS, METHODS, AND TERMINOLOGY

The studies on tobacco were confined to a strain of Wisconsin-Havana Seed (Havana No. 38) unless stated otherwise. The seed was obtained from successive cuttings from a single plant, and precautions were taken to prevent cross-pollination. Tests were carried out from time to time with Turkish tobacco, and while this type had certain advantages, these were offset by disadvantages not possessed by the strain of tobacco used. The studies on *Nicotiana glauca* R. Grah. were confined to a strain collected by the writer on the island of Teneriffe in the Canary group. *N. rustica* L., *N. glutinosa* L., *N. sylvestris* Spegaz. and Comes, and a strain of *N. affinis* Hort. were employed in some of the tests. Seed and young plants of these species were supplied by E. E. Clayton and J. E. McMurtrey.

Unless otherwise stated, the common-mosaic virus used originated from material supplied the writer by James Johnson, the eleventh source recorded in table 1. Previous to the present studies, dilution tests were carried out to determine the power of increase of the virus in tobacco. A series of dilutions was made up to 100,000 in water. Each of these was inoculated into five young tobacco plants. Soon after the appearance of the common-mosaic mottling, virus extract was obtained from one plant in the highest dilution series in which symptoms were expressed. This extract was again diluted in series and inoculated into tobacco plants. This procedure was continued through seven dilution tests. Virus was taken from plants inoculated with extract diluted 10,000 times in 3 tests, and from plants inoculated with extract diluted 100,000 times in 4 tests. It was in the eighth test that all plants with mosaic were allowed to grow to maturity, and it was in these plants that the yellow-mosaic spots were first noted.

During these early tests only the common mosaic was in the greenhouse, and in the first seven tests no yellow-mosaic spots appeared on the young plants before they were discarded. The virus of common mosaic used in all the detailed studies originated from the plants in the eighth dilution test and from leaves which were free of yellow-mosaic spots. Throughout all the work this virus has been guarded to prevent contamination from outside sources, and transfers have been made from tissue which appeared to be free from yellow-mosaic spots, though it is recognized that exceedingly small zones of yellow-mosaic virus not detectable are very likely to be present in some of these tissues.

The mild dark-green mosaic [6] (*15*) used in the tests was isolated from a mixture of mosaics, one of which is a light-green mosaic (common mosaic) which in turn developed yellow-mosaic spots. The original mixture came from mosaic *Nicotiana glauca* plants collected by the writer in the Canary Islands. The mild dark-green mosaic did not develop yellow-mosaic spots in the tests reported in this paper.

[6] Pigment analyses carried out by Peterson (*21*) show that chlorophyll reduction is less in tobacco plants with mild dark-green mosaic than in plants with common mosaic. This relationship is indicated also in the appearance of the plants after mild dark-green mosaic has passed through the mild stage referred to in an earlier paper (*15*). Common mosaic is comparatively light green in color and was referred to as light-green mosaic in another paper (*15*).

However, if plants with this mosaic become reinfected with common mosaic, yellow-mosaic spots may appear before the presence of common mosaic becomes evident. Isolations from such spots have always shown the presence of the common-mosaic virus as well as a yellow-mosaic virus.

Purified yellow mosaics, type A and type B, briefly described in this paper, were also used. The type A yellow mosaic was used in most of the tests as it was isolated from the common mosaic also used throughout the tests. Viruses of these yellow mosaics are referred to as "pure" because present methods fail to demonstrate the presence of other viruses. In perpetuating these mosaics the virus extract was always taken from yellow areas on the leaves, but when large quantities of extract were required entire leaves were used. Several other collections of virus were used in the survey tests, but these were not employed in the detailed studies.

The routine inoculations were made by the needle-cotton method previously described (*14*), unless otherwise stated.

All plants with common mosaic which were used for the yellow-mosaic spot observations were grown to maturity unless the spots appeared earlier. In a few cases plants were cut back when yellow-mosaic spots did not appear before maturity in order to give an opportunity for yellow-mosaic spots to develop on new leaves. This cutting was done with pruning shears; these and the hands of the operator were thoroughly washed in 95-percent alcohol before the pruning of each plant.

Ample uninoculated controls were employed. Pots were sterilized with steam before use. Fertile soil was used and a 1-percent calcium nitrate solution was applied to the plants at intervals during the tests. Soil was checked for the presence of virus by close observation of the healthy plants. The greenhouses used at Madison, Wis., were not screened against insects, but those at the Arlington farm were screened with 30-mesh copper-wire gauze. Fumigations were made at intervals as a safety measure. Plants inoculated with a given virus extract were spaced to prevent their touching plants inoculated with another virus extract, and in certain studies screen partitions were used as previously illustrated (*15*, *fig. 6*). Pots were set on low benches or on sterilized boards on the ground.

During the period of the Allison V. Armour expedition of collection (1926–27) (*15*) many inoculation tests were carried out with tobacco plants grown in pots on the upper deck of the yacht, while on the Atlantic Ocean. In these tests the vector problem was reduced to a minimum.

The routine temperatures of the greenhouses were near 21° to 24° C. in winter unless otherwise stated. During bright days the temperature sometimes went higher. In the summer the temperatures were slightly above those outdoors. The roof and sides of the greenhouses were whitewashed during the spring and summer months.

In this paper the term "mutation" is used to designate the "breaking-up" characteristic of the virus of common mosaic because the evidence to be presented indicates that the new viruses are not contaminants from outside sources and because they represent permanent departures from an established type.

The terms "type" and "strain" are used more or less interchangeably to designateviruses differing from each other irrespective of the

degree of difference. This usage seems to be permissible until there is a more complete agreement among investigators on points relating to the relative importance of virus characteristics and to the usage of terms.

INOCULATION SURVEY OF COLLECTIONS

Mosaic viruses were obtained from 26 sources and tested on Havana Seed tobacco. As indicated in table 1, 23 of these were common mosaic or mosaics resembling common mosaic, and 3 were distinctly different from common mosaic. All plants were closely observed for the development of yellow-mosaic spots, and it was found that, without exception, all plants manifesting common mosaic or a mosaic resembling common mosaic developed one or more yellow-mosaic spots which were either bright yellow or yellow intermingled with green.

It was not possible to make isolations from the yellow-mosaic spots appearing on each plant, but isolations were made from time to time, and without fail, when the spots were large enough to yield sufficient virus, yellow mosaics eventually were established. In the case of very small spots frequently there was insufficient virus to induce yellow mosaic in the inoculated plants.

TABLE 1.—*Inoculation tests with mosaic viruses obtained from different localities and laboratories to determine the occurrence of yellow-mosaic spots*

Type of mosaic on tobacco	Locality where obtained	Collaborator or collector	Plant on which collected	Tobacco plants on which mosaic symptoms developed	Plants which developed yellow-mosaic spots	Places where tests were conducted [1]
				Number	*Number*	
Light-green mosaic (common mosaic).	Connecticut	G. P. Clinton	Tobacco	10	10	A, B
Do	Florida	W. B. Tisdale	do	5	5	B
Do	Louisiana	H. H. McKinney	Tomato	15	15	A, B
Do	do	do	do	5	5	B
Do	Missouri	B. M. Duggar	Tobacco	10	10	A, B
Do	New York	F. M. Blodgett	do	5	5	A
Do	Virginia	H. A. Allard	do	3	3	B
Do	do	do	do	3	3	B
Do	do	Walter Marcey	Garden pepper	5	5	B
Do	do	S. A. Wingard	Tobacco	15	15	A, B
Do	Wisconsin	James Johnson	do	4,224	4,224	A, B, C
Do	do	S. P. Doolittle	do	5	5	B
Do	Hawaii	J. Atherton Lee	do	15	15	A, B
Do	England	A. J. Riker	do	10	10	B
Do	Island of Teneriffe.	H. H. McKinney	*Nicotiana glauca*	15	15	B, C
Do	Island of Grand Canary.	do	do	10	10	B
Resembles common mosaic.	do	do	do	5	5	B
Do	do	do	do	5	5	B
Do	do	do	do	10	10	B
Do	do	do	do	5	5	B
Do	do	do	do	5	5	B
Do	do	do	do	10	10	B
Do	do	do	do	5	5	B
Mild dark-green mosaic.	do	do	do	318	[2] 0	B
Do	Gibraltar	do	do	62	0	B
Mild mosaic	Ephrata, Pa	do	Tobacco	371	[2] 0	B

[1] The letters A, B, and C indicate that the test was conducted at Madison, Wis.; Rosslyn, Va.; or on shipboard, respectively.

[2] Yellow mosaic dominated 3 plants with mild dark-green mosaic and 2 plants with mild mosaic due to accidental contamination.

Healthy plants remained free of these spots. An occasional case of common mosaic and of concentrated yellow mosaic did develop out of the many thousands of healthy tobacco plants grown for various and sundry purposes, but plants used for healthy controls remained free of visible evidence of mosaic.

While all of these collections have not been given intensive study, observations indicate that all collections of mosaic which ordinarily pass for common mosaic on tobacco are not identical in their expression of symptoms on tobacco or on *Nicotiana glauca*. One of the collections from Virginia differs slightly in the mosaic pattern, and the collection from Hawaii has been characterized by a more pronounced expression of the yellow-mosaic spots than other collections.

Three green mosaics which differ from common mosaic were studied on tobacco regularly in the same greenhouses in which the studies on common mosaic and concentrated yellow mosaic were being conducted. The first mosaic in this group is the mild dark-green type. Many of the properties of the virus of this mosaic are similar to those of common mosaic, but it produces no mottling on tomato, and all tests indicate that the tomato plant does not carry the virus. Mild dark-green mosaic has not developed yellow-mosaic spots in the writer's tests. However, of the 138 cases of this disease which were studied, one leaf on one plant was observed to develop a single small light-green mosaic spot. This spot when isolated and inoculated into healthy plants induced a mosaic resembling the mild dark-green mosaic, but light-green semitranslucent spots developed soon after inoculation and the leaves became very deformed, in contrast to the more nearly normal-shaped leaves on plants having the mild dark-green mosaic. The rarity of the occurrence of the light-green spots on plants with mild dark-green mosaic makes it difficult to interpret the origin of this new virus, but it seems reasonable to believe they may originate in the same manner as the yellow-mosaic spots associated with common mosaic.

The second mosaic of this group resembles the first, but differs somewhat in symptoms. It was collected by the writer on *N. glauca* at Gibraltar (*15*). It has not shown the presence of yellow-mosaic spots.

The third mosaic of this group develops mild mottling throughout the life of the plant. It was collected by the writer in a commercial tobacco field at Ephrata, Pa. The virus can be maintained for only a very short period outside the plant, and it is usually more severe on tomatoes than common mosaic; it induces mosaic on Ambalema tobacco, the variety which Nolla et al. (*20*) found to be resistant to common mosaic. This mild mosaic has not developed local spots of yellow mosaic on Wisconsin-Havana Seed tobacco.

These tests suggest that the yellow-mosaic spots are universally associated with common mosaic of tobacco when test conditions are suitable, and they indicate that these spots are not expressed by certain other mosaics, at least under conditions favoring their expression in common mosaic.

ATTEMPTS TO REMOVE PERMANENTLY ALL TRACES OF YELLOW MOSAIC FROM COMMON MOSAIC

TESTS ON TOBACCO WITH VIRUS SUBJECTED TO DILUTIONS AND TO DIFFERENT PHYSICAL AND CHEMICAL TREATMENTS

Tests have indicated that there is not a great deal of difference between the virus of type A yellow mosaic and the virus of common mosaic with respect to dilution in water. The data in table 2 indicate practically no difference, but in the course of many incidental tests it has been observed that the virus of type A yellow mosaic sometimes fails to induce mosaic in all the inoculated tobacco plants when the virus extract is diluted 1,000 times in water, whereas the virus of common mosaic when diluted 1,000 times has always induced mosaic in all of the inoculated tobacco plants.

When fresh extracts containing these viruses were mixed in equal parts the symptoms on inoculated tobacco plants were a blend between those of common and those of yellow mosaic, the tissues that showed the green or common mosaic being in excess of those that showed the yellow mosaic. Mixtures of inoculum which contained the small amounts of the virus extract of yellow mosaic induced less yellow mosaic than mixtures which contained the larger amounts. It will be noted in table 3 that the extracts which contained the smaller proportions of yellow-mosaic virus did not induce blends in all of the plants in a given series. Most of the plants developed only common mosaic during the first few weeks after inoculation, followed by a few of the yellow-mosaic spots characteristic of common mosaic. Mixtures containing 99 or more parts of type A yellow-mosaic extract to 1 part of common-mosaic extract developed intense yellow mosaic soon after inoculation, and the symptoms were like those of the plants which were inoculated with the pure virus of yellow mosaic. As these plants continued their growth common mosaic gradually dominated the new tissues, as indicated in table 4.

TABLE 2.—*Comparative responses of the virus of common mosaic and the virus of type A yellow mosaic to dilution with water, and the numbered leaves showing yellow-mosaic spots on plants with common mosaic, and the approximate size of these spots on each leaf*

Plant no.	No dilution			Diluted 1,000 ×			Diluted 10,000 ×			Diluted 50,000 ×			Diluted 100,000 ×		
	Common mosaic		Yellow mosaic (disease present or absent)	Common mosaic		Yellow mosaic (disease present or absent)	Common mosaic		Yellow mosaic (disease present or absent)	Common mosaic		Yellow mosaic (disease present or absent)	Common mosaic		Yellow mosaic (disease present or absent)
	Disease present or absent	Leaves on which yellow-mosaic spots appeared [1] / Approximate mean diameter of yellow-mosaic spots [2]		Disease present or absent	Leaves on which yellow-mosaic spots appeared [1] / Approximate mean diameter of yellow-mosaic spots [2]		Disease present or absent	Leaves on which yellow-mosaic spots appeared [1] / Approximate mean diameter of yellow-mosaic spots [2]		Disease present or absent	Leaves on which yellow-mosaic spots appeared [1] / Approximate mean diameter of yellow-mosaic spots [2]		Disease present or absent	Leaves on which yellow-mosaic spots appeared [1] / Approximate mean diameter of yellow-mosaic spots [2]	
1	+	20, 35 / 1, 2	+	+	16, 20 / 9, 2	+	+	40, 41 / 5, 5	+	0	---------- / ----------	0	0	---------- / ----------	0
2	+	45 / 2	+	+	18, 25, 32, 33 / 7, 1, 3, 5	+	+	18, 31, 32 / 4, 1, 5	0	0	---------- / ----------	0	0	---------- / ----------	0
3	+	30 / 5	+	+	41 / 5	+	0	---------- / ----------	+	0	---------- / ----------	0	+	10, 40 / 3, 6	+
4	+	5, 30, 37 / 1, 7, 11	+	+	19, 31 / 3, 4	+	0	---------- / ----------	+	+	29 / 12	0	0	---------- / ----------	0
5	+	32 / 3	+	+	42 / 1	+	0	---------- / ----------	+	0	---------- / ----------	0	0	---------- / ----------	0
6	+	41 / 2	+	+	11, 30 / 4, 4	+	+	30 / 4	+	+	6, 29, 32 / 1, 1, 3	0	0	---------- / ----------	0
7	+	12 / 3	+	+	27 / 2	+	0	---------- / ----------	0	0	---------- / ----------	0	0	---------- / ----------	0
8	+	12, 21 / 3, 4	+	+	13 / 1	+	0	---------- / ----------	0	0	---------- / ----------	+	0	---------- / ----------	0
9	+	6, 3, 4 / 3	+	+	9 / 3	+	0	---------- / ----------	0	0	---------- / ----------	0	0	---------- / ----------	0
10	+	14, 20, 21 / 3	+	+	43 / 5	+	+	15, 19 / 5, 7	+	0	---------- / ----------	0	0	---------- / ----------	0

[1] The numbers in this column indicate which leaves developed yellow-mosaic spots. The number 20, for example, signifies the twentieth leaf. The numbering starts with the first leaf to show common mosaic.

[2] The figures in this column indicate the approximate area of the yellow-mosaic spots on each leaf on each plant in terms of the approximate mean diameter in millimeters.

TABLE 3.—*Types of symptoms produced on tobacco when inoculated with synthetic mixtures of virus extracts of common mosaic and of type A yellow mosaic and with each virus separately*

[Inoculations made June 22, 1927; 5 plants inoculated with each extract]

Parts of virus extracts used in the mixtures		Notes taken July 8	Notes taken July 26	Notes taken Aug. 8
Virus extract of common mosaic	Virus extract of yellow mosaic			
1	0	5 plants common mosaic.	5 plants had common mosaic, 1 to 3 yellow-mosaic spots on each plant.	5 plants had common mosaic, with 1 to 4 yellow-mosaic spots on each plant.
999	1	5 plants common mosaic, but 1 plant had yellow-mosaic spots.	New leaves on 5 plants had common mosaic with 1 or 2 yellow-mosaic spots per plant.	New leaves on 5 plants had common mosaic with 1 to 3 yellow-mosaic spots per plant.
499	1	5 plants common mosaic, but 2 plants had yellow-mosaic spots.	New leaves on 5 plants had common mosaic with 1 to 3 yellow-mosaic spots per plant.	New leaves on 5 plants had common mosaic with 1 to 4 yellow-mosaic spots per plant.
99	1	do	New leaves on 5 plants had common mosaic with 1 to 4 yellow-mosaic spots on each plant.	Do.
1	1	5 plants common and yellow mosaic, but common mosaic predominated.	5 plants had many yellow-mosaic spots on new leaves, but common mosaic predominated.	New leaves on 5 plants had common mosaic with 1 or 2 yellow-mosaic spots per plant.
1	99	5 plants had symptoms of yellow mosaic only. Looked like yellow-mosaic control.	5 plants had severe yellow mosaic, but more green tissue than yellow-mosaic control.	Do.
1	499	do	5 plants looked like the yellow-mosaic controls.	All 5 plants had much yellow mosaic, but common mosaic was showing in top leaves of 5 plants.
1	999	do	do	All plants had much yellow mosaic, but common mosaic was showing in top leaves of 4 plants.
0	1	5 plants had symptoms of yellow mosaic only.	5 plants had symptoms of pure yellow mosaic.	5 plants had symptoms of pure yellow mosaic in all of the leaves.

TABLE 4.—*Progressive change in type of symptoms on tobacco leaves as the plants developed*

Observations were made on each plant which was inoculated with the virus mixture containing 1 part of extract of common mosaic and 99 parts of extract of type A yellow mosaic in the experiment cited in table 3. Notes were taken Aug. 8. Leaf numbers start with the first leaf to show symptoms after inoculation]

Type of symptoms on leaves	Leaf numbers in each symptom group on each plant				
	Plant 1	Plant 2	Plant 3	Plant 4	Plant 5
Dead; originally all yellow mosaic	1–5	1–4	1–5	1–5	1–4
Entirely yellow mosaic	6–13	5–16	6–13	6–13	5–11
Blends of common mosaic and yellow mosaic	14–20	17–22	14–21	14–19	12–16
Common mosaic with the occasional yellow-mosaic spots	21–27	23–26	22–28	20–28	17–27

In an earlier paper (*15*) it was pointed out that tobacco plants having common mosaic show no changes in symptoms after being reinoculated with the pure undiluted virus of yellow mosaic. The same results have been obtained with all the yellow mosaics which

the writer has isolated from tobacco and tested against common mosaic. Thung (*27*) later reported similar observations, and he reports that tobacco plants having the yellow or "white" mosaic show no change in symptoms when reinoculated with the virus of common mosaic. The writer's results are not in accord with those of Thung on this point. When plants with type A yellow mosaic growing at temperatures of about 21° to 24° C. were reinoculated with a fresh undiluted virus extract of common mosaic, from 5 to 20 of the subsequent leaves exhibited yellow mosaic, the characteristic green mottling of common mosaic then gradually appeared, and from 8 to 25 leaves developed blended symptoms, the green mottling of common mosaic gradually gaining in proportion to the yellow. The subsequent leaves exhibited the characteristic mottling and the occasional small yellow-mosaic spots typical of common mosaic. Virus from light- and dark-green mottled portions of these leaves induced common mosaic when inoculated into healthy tobacco plants. When the stems of mature plants were cut back leaving only the portions which had developed the yellow-mosaic leaves prior to the reinoculation, it was found that the buds produced branches which developed typical common mosaic. Yellow-mosaic control plants which were not reinoculated exhibited typical yellow mosaic throughout the test, and the lower side shoots developed typical yellow mosaic when the stems were cut back. Thung may have discarded his test plants too soon; also it is possible that his method, his viruses, his tobacco, or the environment may account for his negative results.

From the results presented in the foregoing paragraphs, it seems reasonable to conclude that the virus of yellow mosaic either is not present in available form in tissues showing only the common green mosaic or if it is present the number of virus particles is considerably smaller than the number of particles of common-mosaic virus. Therefore, on diluting a virus extract obtained from leaves showing only the common green mosaic, a point should be reached where the number of particles of the yellow-mosaic virus would be nil or too small to produce infection, but where there would be a sufficient number of particles of the other virus to induce infection. On the basis of random assortment, a high percentage or all of the infected plants resulting from such a dilute virus should develop the common green mosaic free from yellow-mosaic spots.

Dilution tests were carried out and the plants were held for close observation throughout the entire life cycle. Plants which developed common mosaic also developed yellow-mosaic spots, and there was no essential difference in the relative time of their appearance or in the range in number or size of the spots produced on plants inoculated with dilute virus as compared with plants inoculated with concentrated virus. The results of a typical test are shown in table 2. These results were unexpected, for it was thought that the virus of yellow mosaic could be readily eliminated from the virus of common mosaic. The phenomenon appeared to represent some unusual type of mixture or a mutation of the common-mosaic virus. With these possibilities in mind further tests were carried out.

It was found that dilute virus of common mosaic was inactivated at slightly lower temperatures than concentrated virus (*13*, *22*). Accordingly extracts were diluted 1,000 times in water and treated for 10 minutes at temperatures ranging from 85° to 90° C. All the

inoculated plants that developed symptoms, first expressed the green mottling typical of common mosaic, which was indistinguishable from the symptoms in plants inoculated with the undiluted unheated virus. As the plants developed, the typical yellow-mosaic spots appeared on from 1 to 6 leaves on each plant, and the symptoms in the plants inoculated with the heated virus were essentially indistinguishable from those in the plants inoculated with the unheated virus.

In view of the previous results it seemed strange that these treatments failed to eliminate the yellow-mosaic virus if it represented a mixture. Accordingly, especial attention was given to methods for preventing the possible accidental contamination of the experimental materials.

As the studies progressed and it was found that the purified mild dark-green mosaic collected in the Canary Islands failed to manifest yellow-mosaic spots, it became evident that this mosaic might prove of value in testing for the separation of yellow-mosaic virus from it in synthetic mixtures. When these viruses were blended in different proportions the plants inoculated with mixtures containing large proportions of virus of yellow mosaic developed only the symptoms typical of pure yellow mosaic. When the proportions of virus of mild dark-green mosaic were increased some of the inoculated plants developed only yellow mosaic, some expressed blended symptoms followed by intense yellow mosaic, and some showed symptoms of mild dark-green mosaic with no signs of yellow mosaic. The results of two tests with mixtures including type A yellow-mosaic virus are presented in table 5.

TABLE 5.—*Types of symptoms expressed by tobacco plants inoculated with synthetic mixtures of virus extracts of mild dark-green mosaic and type A yellow mosaic, and with each virus separately* [1]

TEST 1

Plant no.	GM only	1 part GM, 1 part YM	9 parts GM, 1 part YM	49 parts GM, 1 part YM	99 parts GM, 1 part YM	249 parts GM, 1 part YM	YM only
1	G	Y	Y	GY	G	G	Y
2	G	Y	Y	GY	GY	G	Y
3	G	Y	Y	GY	GY	G	Y
4	G	Y	Y	GY	GY	G	Y
5	G	Y	Y	GY	GY	Y	Y

TEST 2

Plant no.	GM only	1 part GM, 1 part YM	9 parts GM, 1 part YM	99 parts GM, 1 part YM	499 parts GM, 1 part YM	999 parts GM, 1 part YM	YM only
1	G	Y	Y	Y	GY	Y	Y
2	G	Y	Y	GY	GY	GY	Y
3	G	Y	GY	GY	GY	GY	Y
4	G	Y	GY	GY	GY	GY	Y
5	G	Y	GY	GY	GY	G	Y

[1] GM and YM in the box heads indicate virus extract from mild dark-green mosaic and from yellow mosaic, respectively. G and Y in the columns indicate the symptoms of mild dark-green and of yellow mosaic, respectively, in the plants that were inoculated with the virus mixtures or with pure viruses as indicated. In the case of all plants that developed the symptoms of both mosaics, the symptoms of green mosaic eventually were lost and only the yellow mosaic was expressed by the new leaves.

Healthy tobacco plants and tomato plants were inoculated with virus obtained from plant no. 5 in table 5, test 2, which had been inoculated with 999 parts of extract from mild dark-green mosaic to 1 part of extract from yellow mosaic. Tomatoes are not susceptible to this green mosaic, but are very susceptible to type A yellow mosaic. The tomato plants remained free of mosaic and the tobacco plants developed mild dark-green mosaic with no signs of yellow-mosaic spots.

It was pointed out in another paper (*15*) that plants with mild dark-green mosaic when reinoculated with the virus of yellow mosaic ultimately developed yellow mosaic on the new foliage, the reverse of the situation in common mosaic. Blended symptoms developed first, followed later by yellow mosaic which crowded out the mild dark-green mosaic. Plants showing the blended stage in the symptoms frequently produced leaves with rather large areas which were devoid of yellow-mosaic spots. Such areas were used to supply virus for inoculating healthy tobacco plants. In one test five healthy plants were inoculated with undiluted extract from such tissue and all plants developed yellow mosaic. The same extract was diluted to 1,000 times its original volume in sterile distilled water and used to inoculate five healthy tobacco plants. Each plant developed mild dark-green mosaic and each was free from yellow-mosaic spots throughout the life cycle.

When the virus of type B yellow mosaic was mixed with the virus of mild dark-green mosaic in the proportion of 1 part to 99 parts, only 1 plant of the 10 inoculated developed mild dark-green mosaic and showed no trace of yellow mosaic. The results of this test are presented in table 6.

TABLE 6.—*Expression of green mosaic, yellow mosaic, or blends of the two, by tobacco plants inoculated with synthetic mixtures of virus extracts of mild dark-green mosaic and type B yellow mosaic and with each virus separately* [1]

Plant no.	GM only	99 parts GM, 1 part YM	249 parts GM, 1 part YM	YM only	Plant no.	GM only	99 parts GM, 1 part YM	249 parts GM, 1 part YM	YM only
1	G	GY	GY	Y	6		GY	GY	
2	G	GY	GY	Y	7		GY	GY	
3	G	GY	GY	Y	8		GY	GY	
4	G	GY	GY	Y	9		GY	GY	
5	G	GY	GY	Y	10		G	GY	

[1] GM and YM in the box heads indicate virus extract from mild dark-green mosaic and from yellow mosaic, respectively, in the inoculum. G and Y in the columns indicate the symptoms of mild dark-green and of yellow mosaic, respectively, in the plants that were inoculated with the virus mixtures or with pure viruses as indicated. In the case of all plants that developed the symptoms of both mosaics, the symptoms of green mosaic eventually were lost and only the yellow mosaic was expressed by the new leaves.

The results obtained with the mixtures of mild dark-green mosaic and the yellow mosaics are in accord with the results expected from ordinary mixtures or contaminations in which an independent random distribution of the units or particles occurs. The fact that yellow mosaics could be eliminated from the mild dark-green mosaic without resorting to extremely high dilutions or special treatments, but could not be eliminated from the diluted virus of common mosaic, is a strong indication that the virus of the yellow-mosaic spots does not constitute an ordinary contamination from an outside source, but arises in the plant after the onset of common mosaic.

While these tests strongly suggested that the virus of yellow mosaic represented a mutant, it seemed advisable to give consideration to the hypothesis that this virus has an inactive stage caused by adsorption or some other phenomenon, and that the yellow-mosaic spots develop when something happens to activate the virus. If this hypothesis is correct, in accordance with the theory of random sampling from a mass in which there is independent random distribution, the dilution tests should have eliminated the inactive forms, at least in a few of the plants, which would result in some cases of common mosaic without the yellow spots, unless the inactive particles are always greatly in excess of those of the common-mosaic virus. If this is true the dilution studies again should supply the evidence.

With dilutions of common mosaic of 50,000 to 100,000 times, some of the many plants which show no common mosaic should have developed late signs of yellow mosaic as a result of inactive yellow-mosaic particles which survived these dilutions, such particles later becoming active and producing infection. Yellow-mosaic spots such as are associated with common mosaic probably would not occur on these plants since the suppressing influence of the common green-mosaic virus would be absent. The yellow-mosaic symptoms in such plants should be comparable to those which develop when healthy plants are inoculated with pure virus of this mosaic. Of the many plants observed throughout the dilution studies, no such case has ever occurred. These results seemed to give satisfactory grounds for rejecting the hypothesis advanced in the preceding paragraph, but further tests were carried out to determine whether some treatment of the virus of common mosaic in vitro might shed light on the possibility that some type of dissociation phenomenon was operating. If such were the case striking blends of yellow and green mosaic, pure green mosaic, and pure yellow mosaic should occur in a population of plants inoculated with virus given certain treatments.

Extracts were obtained from leaves showing common mosaic without the yellow-mosaic spots. These extracts, in diluted and in undiluted form, were subjected to high temperatures (85° to 90° C.), to subfreezing, and to ultraviolet rays. Extracts were filtered and also treated with several concentrations of acid, alkali, acetone, and ethyl alcohol. Some of the extracts were first purified to a point where only slight traces of the original extraneous solids, salts, and soluble pigments were present in a water suspension of the virus. In all cases of common mosaic which resulted from virus receiving these treatments, the delayed expression of a few small yellow-mosaic spots occurred, no plants developed pure yellow mosaic, and none developed yellow-mosaic spots without the common mosaic. While many other tests might have been devised, it seemed useless to carry such methods any farther. None of the results presented point to the view that the virus of yellow mosaic is in an active or in an inactive form in each and every experimental extract of common-mosaic virus used as inoculum in the tests, but rather they favor the view that the yellow-mosaic virus arises spontaneously as a mutant.

Another line of evidence against the mixture theory is found in the results presented in table 3 and on page 962. It will be recalled that these results indicate that the viruses of type A yellow mosaic and of common mosaic are not mutually compatible in tobacco. The virus

of common mosaic is the more aggressive, as it gradually suppresses or inhibits the virus of yellow mosaic which is introduced in synthetic mixtures. This inhibiting characteristic is taken up further in the final discussion on acquired immunity, and while the phenomenon is not fully understood, it seems quite clear that it militates against or prevents the indefinite survival of the yellow-mosaic viruses thus far tested which were introduced experimentally into the virus of common mosaic. All available evidence obtained at temperatures near 21° to 24° C. indicates that unless there is a considerable quantity of yellow-mosaic virus present with the virus of common mosaic in an inoculum, the inoculated plants express symptoms of common mosaic which are indistinguishable from the symptoms expressed by the controls inoculated with the purest virus of common mosaic available (table 8). If there is sufficient virus of yellow mosaic in such a mixture to induce mild signs of yellow mosaic, these signs appear before, or simultaneously with, the first signs of common mosaic, but the subsequent leaves manifest symptoms like the controls, i. e., light- and dark-green mottling with occasional yellow-mosaic spots.

The inhibiting characteristic of the common-mosaic virus is regarded as one of the strongest bits of evidence in support of the view that the occasional yellow-mosaic spots which are persistently associated with common mosaic under the conditions of the writer's tests result not from virus introduced as a contaminant from the outside but from virus which originates as a mutant in the tissues involved in the spots. In fact, the results of all the tests presented thus far seem to support this view.

TESTS WITH A DIFFERENTIAL PLANT WHICH DEVELOPS SYSTEMIC INFECTION

It has been known for some time that certain viruses can be separated from mixtures by means of plants which are resistant to one virus but not to another. In these studies it was found that a strain of *Nicotiana glauca* served as a differential plant for the viruses of type A yellow mosaic and common mosaic in a rather unique manner at temperatures from about 21° to 24° C. in the greenhouse. When inoculated with these viruses the plants rarely developed symptoms of mosaic. When symptoms did develop they were very mild, and in the case of the yellow mosaic a few mild yellow spots occurred on 1 or 2 leaves only near the point of inoculation, the new leaves remaining mosaic-free.

It was found that a very high percentage of the *Nicotiana glauca* plants which were inoculated with the common-mosaic virus were carriers of this virus throughout their life, even though the plants manifested no visible signs of mosaic. Allard (*1*) reported similar results with *N. glauca* and common mosaic. In contrast with these results, it was found that none of the *N. glauca* plants which had been inoculated with the virus of type A yellow mosaic carried the virus of that mosaic in mosaic-free leaves which developed after inoculation. Thirty-five *N. glauca* plants were inoculated with the virus of type A yellow mosaic and tested in this manner, and all the tobacco plants subinoculated from them remained free of all signs of mosaic. The results of a single experiment showing the differential relationship between the viruses of common mosaic and the yellow mosaic on *N. glauca* are given in table 7.

TABLE 7.—*Behavior of Nicotiana glauca plants when inoculated with virus of common mosaic in contrast with plants inoculated with virus of type A yellow mosaic, and the presence or absence of virus in the extracts from upper leaves of each N. glauca plant as shown by inoculating the extracts into tobacco plants*

Virus of common mosaic used				Virus of type A yellow mosaic used			
Plant no.	Presence or absence of mosaic on each *N. glauca* plant	Presence or absence of virus in each *N. glauca* plant as evidenced by plants inoculated with extracts from the upper leaves of each *N. glauca* plant 96 and 266 days after the inoculation of the latter [1]		Plant no.	Presence or absence of mosaic on each *N. glauca* plant	Presence or absence of virus in each *N. glauca* plant as evidenced by plants inoculated with extracts from the upper leaves of each *N. glauca* plant 96 and 266 days after the inoculation of the latter [1]	
		96 days	266 days [2]			96 days	266 days [2]
1	−	[3]+ +	[3]+ +	1	−	− −	− −
2	−	+ +	+ +	2	−	− −	− −
3	−	+ +	+ +	3	−	− −	− −
4	−	+ +	+ +	4	−	− −	− −
5	−	+ +	+ +	5	−	− −	− −
6	−	+ +	+ +	6	−	− −	− −
7	−	+ +	+ +	7	−	− −	− −
8	−	+ +	+ +	8	−	− −	− −
9	−	+ +	+ +	9	−	− −	− −
10	−	+ +	+ +	10	[4]s	− −	− −

[1] Extracts were obtained from nearly full-sized leaves near the top of each plant of *N. glauca*.
[2] All plants of *N. glauca* were cut back and new shoots were allowed to develop and supply the extracts used in the second test on tobacco.
[3] From 1 to 5 small yellow-mosaic spots eventually appeared on each tobacco plant that developed common mosaic.
[4] 1 small yellow-mosaic spot appeared on 1 leaf slightly above the point of inoculation.

In another test a mixed extract obtained from equal parts of common mosaic and type A yellow-mosaic leaf tissues from tobacco was inoculated into a *Nicotiana glauca* plant. Small yellow-mosaic spots and yellowish-green spots developed on one leaf, but no other symptoms appeared on the plant. Thirty-five days after this plant was inoculated five healthy tobacco plants were inoculated with a virus extract from these spots. All of the tobacco plants developed yellow mosaic. At the same time an extract was obtained from mosaic-free leaves above the leaf with yellow-mosaic spots. This virus extract was inoculated into five healthy tobacco plants, all of which developed only common mosaic. No blends with yellow mosaic occurred, but before the plants were mature a few of the characteristic yellow-mosaic spots developed on each of them. A similar experiment was carried out with a mixture of the viruses of type A yellow mosaic and the mild dark-green mosaic. Symptoms of the mild dark-green mosaic appeared on all the plants. The healthy tobacco plants which were finally inoculated with virus from the upper leaves of these *N. glauca* plants developed only the mild dark-green mosaic; no yellow mosaic developed on any of them.

These results show that the virus of type A yellow mosaic was local in the strain of *Nicotiana glauca* used when it was grown under the conditions of the tests, and that leaves above this local region served a positive filterlike function against the virus of the yellow mosaic which was introduced in the inoculum. It will be noted in table 7 that all of the tobacco plants which were inoculated with the common-mosaic virus taken from *N. glauca* developed common mosaic.

Before maturity from 2 to 5 small yellow-mosaic spots appeared on each of the plants having common mosaic. Since no virus of yellow mosaic was recovered from the upper leaves of *N. glauca* plants which had been inoculated with virus of type A yellow mosaic, it is concluded that any virus of type A yellow mosaic which might by chance have been present in the inoculum of common mosaic used to inoculate *N. glauca*, did not reach the upper leaves of these plants. Therefore, since the few yellow-mosaic spots were delayed in their appearance on each tobacco plant, it is concluded further that the virus which induced the yellow-mosaic spots in the test tobacco plants originated in the tissue of these spots. These results are in agreement with those obtained by other methods in that they indicate virus mutation in common mosaic.

VIRUS ISOLATIONS FROM LOCAL NECROTIC LESIONS AND PRELIMINARY STUDIES ON THE LESION TECHNIQUE

The necrotic-lesion technique devised by Holmes (*6*) was used to throw additional light on the problem of virus mutation, and the results were reported briefly in an earlier résumé (*19*).

Holmes (*6*) suggested that necrotic lesions might be analogous to the colonies of bacteria obtained in the plating and purifying technique. Accordingly the writer used the method to determine the possibility of reclaiming the virus of mild dark-green mosaic from synthetic mixtures with the virus of type A yellow mosaic. This was done as a preliminary step as it seemed reasonable to believe the method could be applied to other viruses if satisfactory results were obtained.

Leaves of *Nicotiana glutinosa* were removed from the plants, wiped with virus extract, and thoroughly rinsed with sterile distilled water. The leaves were then placed in a previously sterilized covered dish and kept in a humid atmosphere at about 22° C. until the necrotic lesions developed, great care being taken to maintain aseptic conditions, so far as foreign viruses were concerned, during the whole procedure. The lesions were examined under a lens in order to discard any cases in which incipient lesions might be close to the typical lesions. Lesions sufficiently separated were carefully removed by means of a small sharp dissecting spear, and each lesion was macerated and pushed into the junction point of a petiole and stem of a mosaic-free tobacco plant.

Nineteen lesions were selected at random from leaves wiped with the above-mentioned mixture of viruses. Each lesion was used to inoculate a single tobacco plant. Two of these plants developed yellow mosaic, 6 developed typical mild dark-green mosaic with no signs of yellow mosaic, and the remaining 11 were mosaic-free. Since the virus of mild dark-green mosaic was recovered free from the virus of yellow mosaic, it was concluded that this method serves to separate mixed viruses.

Another test was carried out with the virus of common mosaic. This extract was obtained from tobacco leaves showing no signs of yellow-mosaic spots. It was diluted in water to reduce the number of lesions and used to wipe the leaves removed from *Nicotiana rustica* and *N. glutinosa* plants. One hundred and twenty-seven lesions were isolated from these leaves and inoculated separately into the same number of young tobacco plants. Of these plants, 120 developed

typical common mosaic, and typical yellow-mosaic spots eventually made their appearance on each plant. The 7 mosaic-free plants remained so during the test. The appearance of the yellow-mosaic spots was delayed, and 1 to 3 spots appeared on each plant as is usual in common mosaic at moderate growing temperatures.

As in all previous tests, the results obtained with the local-lesion technique indicate that the virus of the yellow-mosaic spots does not represent a contamination from outside sources which is carried along in all inoculation extracts of common mosaic, and they support the idea of virus mutation. This conclusion is based on the successful separation of the virus of mild dark-green mosaic from the virus of yellow mosaic, and not on the assumption that necrotic lesions are incited by single virus particles.

This paper deals primarily with the problem of virus mutation and not with the necrotic-lesion technique. However, since this method is used in virus-mutation studies, it seems permissible to digress from the main subject long enough to discuss certain aspects of this technique.

It has been considered (*8, 10, 23*) that the local lesions rarely contain more than one type of virus when mixed viruses are used to wipe the leaves of suitable species, and it is inferred that the lesions are induced for the most part by single virus particles. So far as the writer knows, no data have been presented which make it possible to pass fair judgment on this point. Mixed extracts containing the viruses of common mosaic and yellow mosaic have been used to wipe leaves of species which produce local necrotic lesions. The resulting necrotic lesions have been isolated and each has been inoculated into a single tobacco plant, with the result that the infected plants developed either yellow mosaic or common mosaic, but rarely blended symptoms of both diseases. On this basis it has been considered that necrotic lesions are rarely incited by more than a single virus particle.

This method of determining the presence of type A yellow-mosaic virus in a lesion which also contains the virus of common mosaic is of questionable value for the reason that all the evidence indicates that the virus of common mosaic is more aggressive than that of type A yellow mosaic. It is admitted that this evidence was obtained from systemic symptoms, but until otherwise shown, it is reasonable to suppose that the same aggressive relationship obtains in lesions.

The data presented in table 3 rather clearly indicates that this aggressive characteristic prevented the expression of yellow-mosaic symptoms in many tobacco plants which were inoculated with yellow-mosaic virus extract that had been diluted 100, 500, and 1,000 times in virus extract of common mosaic. All the plants that were inoculated with mixtures containing the virus extract of yellow mosaic in quantities greater than the virus extract of common mosaic first developed the symptoms of pure yellow mosaic, and these persisted for some time. During this period the experimenter would conclude that these plants had received no virus of common mosaic in the inoculum, and if the plants had been discarded during this period it is evident from the tables that this conclusion would be erroneous. On long standing, each of these plants eventually gave the true story. Even though the quantity of common-mosaic virus was smaller in the

beginning, its aggressive and suppressive characteristics made possible the manifestation of common mosaic before the plants were mature.

The tests cited in tables 3 and 4 were made some time before the local-lesion method was developed. However, after Kunkel (*11*) reported that *Nicotiana sylvestris* developed necrotic lesions when inoculated with virus of aucuba mosaic (a yellow mosaic), but systemic symptoms when inoculated with the virus of common mosaic, the following tests were carried out. These tests were made to determine further the possibility of getting a satisfactory assay for yellow-mosaic virus in mixtures with the virus of common mosaic when using tobacco as the test plant.

Fresh extracts were obtained from actively growing leaves on tobacco plants with type A yellow mosaic and also from plants with common mosaic. These extracts were mixed in the proportion of 1 part to 99 parts and 1 part to 999 parts, respectively. In addition, there were controls in which water was used to dilute the virus of yellow mosaic. Each of these extracts, including the undiluted extracts, was used to inoculate 10 healthy tobacco plants by the needle-cotton method described previously (*14*), to inoculate 10 healthy tobacco plants by wiping 1 medium-sized leaf on each plant, and to wipe 10 or more leaves on *Nicotiana sylvestris* plants. The total leaf area wiped in a given set of 10 tobacco plants was estimated to be about equal to the total leaf area wiped on the *N. sylvestris* plant inoculated with the corresponding extract. The leaf area wiped on each plant of *N. sylvestris* averaged approximately 1,000 cm^2. The leaves of *N. sylvestris* were wiped after the tobacco plants were inoculated.

The tests on *Nicotiana sylvestris* (table 8) show that virus of yellow mosaic was potent in the mixtures with common-mosaic virus which were inoculated into the tobacco plants, and the tests on tobacco with the yellow-mosaic virus which was diluted in water show a high percentage of infection. It will be observed further that no striking case of yellow mosaic appeared in any of the tobacco plants inoculated with the extracts containing the viruses of both mosaics. A few plants showed yellow-mosaic spots on one or more of the first leaves. The great majority of plants developed common mosaic which was indistinguishable from that in the common-mosaic controls. Dilutions of 100 and 1,000 are not great, yet these tests demonstrated that it was not possible by the systemic-symptom method alone to obtain an adequate test for the yellow-mosaic virus which was present in appreciable amounts with the virus of common mosaic. However, subsequent tests have shown that type A yellow-mosaic virus which was present in similar plants with common mosaic was detectable by means of necrotic lesions on the leaves of *N. sylvestris* which had been wiped with the extracts from such plants.

Kunkel (*10*) used *Nicotiana sylvestris* as a test plant for viruses from lesions produced on leaves of *N. langsdorffii* Schrank., which had been punctured with needles carrying mixed viruses of common mosaic and aucuba mosaic. No data were presented, but from these tests he concluded that except in a small percentage of cases the lesions resulted from a single unit of virus. His use of the term "unit" is not defined, but one is left to infer that it signifies one particle and not a minimum dose, which might include several particles.

TABLE 8.—*Results of 2 tests illustrating the uncertainty of determining virus mixtures of common mosaic and type A yellow mosaic at 21° to 24° C.*

Parts of virus extract or water used in preparing the mixtures			Method used for making assay [1]	Notes on tobacco plants based on observations made during the first 30 days after inoculation
Water	Extract of common mosaic	Extract of yellow mosaic		
0	0	1	A	10 tobacco plants had yellow mosaic.
			B	Do.
			C	2.91 necrotic lesions per cm² of leaf surface on *Nicotiana sylvestris*.
99	0	1	A	10 tobacco plants had yellow mosaic.
			B	Do.
			C	0.117 necrotic lesions per cm² of leaf surface on *N. sylvestris*.
0	99	1	A	6 tobacco plants had common mosaic only; 4 tobacco plants had common mosaic predominating, but yellow-mosaic spots appeared on from 1 to 4 of the first leaves to show mottling.
			B	6 tobacco plants had common mosaic only; 4 tobacco plants had common mosaic predominating, but yellow-mosaic spots appeared on from 1 to 3 of the first leaves to show mottling.
			C	0.096 necrotic lesions per cm² of leaf surface on *N. sylvestris*.
0	1	0	A	10 tobacco plants had common mosaic.
			B	Do.
			C	No necrotic lesions on leaf surface of *N. sylvestris*.
0	0	1	A	10 tobacco plants had yellow mosaic.
			B	Do.
			C	1.03 necrotic lesions per cm² of leaf surface on *N. sylvestris*.
999	0	1	A	6 tobacco plants had yellow mosaic; 4 tobacco plants mosaic-free.
			B	10 tobacco plants had yellow mosaic.
			C	0.014 necrotic lesions per cm² of leaf surface on *N. sylvestris*.
0	999	1	A	10 tobacco plants had common mosaic.
			B	10 tobacco plants had common mosaic, but 1 plant had 1 yellow-green spot on the second leaf which developed common mosaic.
			C	0.022 necrotic lesions per cm² of leaf surface on *N. sylvestris*.
0	1	0	A	10 tobacco plants had common mosaic.
			B	Do.
			C	0.006 necrotic lesions per cm² of leaf surface on *N. sylvestris*.[2]

[1] 3 methods of testing were used as indicated by the roman letters as follows: A, Tobacco plants were inoculated by the needle-cotton method previously described (*14*); B, tobacco plants were inoculated by wiping a leaf with the same extract used in method A; C, 10 or more leaves of *N. sylvestris* plants were inoculated by wiping with the same extract used in method A. The number of necrotic lesions is based on a unit area of the leaf surface, i. e., 1 cm².

[2] The common-mosaic tissue used for this control contained a few yellow-mosaic spots.

The writer has not had an opportunity to test the amount of increase of virus in the lesions produced by *Nicotiana langsdorffii*. However, tests have indicated that the increase frequently is very small in necrotic lesions produced by *N. rustica*, *N. glutinosa*, and *N. sylvestris*. Caldwell (*2*) also found a small increase of the virus in the necrotic lesions he tested. It is a common occurrence to get negative results in testing for the presence of virus in necrotic lesions, and with common-mosaic virus it has been unusual to obtain more than 10 lesions on a leaf of *N. rustica* when wiped with extract from a single lesion isolated from *N. rustica*, the leaves in both instances being attached to the plant. This situation makes it difficult to obtain a positive test for the presence of one or the other virus in a mixture if one virus is considerably in excess of the other or if one is decidedly more aggressive than the other. Tests indicate that the central areas in lesions contain little or no virus when they become necrotic; thus in many cases the evidence of a primary mixture might not be obtained when only the peripheral virus is available for test.

If it is found that necrotic lesions in the great majority of cases are incited by single virus particles, it will be of considerable experimental importance. However, wholly aside from the point as to the actual number of virus particles which incite a lesion, it is reasonably evident

that certain necrotic lesions either contain but one type of virus, or in some cases of mixture, one virus is of such high relative concentration that the presence of others in the lesion cannot be detected by ordinary methods. Consequently those in the minority which are not too aggressive are completely lost in series transfers, leaving the most concentrated virus to survive in a pure state.

While the lesion method does permit the separation and purification of certain viruses, it should be made very clear that the techniques which are based on the systemic invasion of the virus and mosaic mottling have made it possible to separate and purify many viruses through the isolation of local tissues which exhibit departures in symptoms, followed by the dilution of the virus extract thus obtained and its subsequent inoculation into populations of plants. This procedure is based on the elementary principle of independent random distribution of the particles in an extract and on reducing the concentration of the virus that is present in smaller quantity to a point where it is no longer detectable in all of the random samples used for inoculating the test population. Through a careful study of symptoms the worker learns to detect irregularities in local areas of tissue in a given plant and in individual plants in a series. Selections and inoculations from these through successive tests frequently establish new types of virus. This method has wide application and can be used in studying certain viruses not known to induce the local lesions.

TESTS WITH DIFFERENTIAL PLANTS WHICH DEVELOP LOCAL NECROTIC LESIONS

In studies on *Nicotiana* species a collection of *N. affinis* was received which comprised several rather obvious genetic types differing in flower color, time of flowering, and texture of leaves. In the group which produced flowers late it was found that necrotic lesions developed on the detached leaves which were wiped with the virus of type A yellow mosaic. In certain of these plants necrotic lesions developed on detached leaves which were wiped with virus of common mosaic, but in other plants the wiped leaves developed yellowish-green areas. Kunkel (*11*) has reported that the virus of common mosaic fails to induce necrotic lesions, whereas the virus of aucuba mosaic (a yellow mosaic) does induce necrotic lesions on the leaves of *N. sylvestris*.

This differential relationship in *Nicotiana affinis* and *N. sylvestris* was used in testing the virus-mutation theory in the following ways. Plants were first tested to determine whether their leaves were negative for the expression of the characteristic necrotic lesions when wiped with virus extract of common mosaic. This was done with detached leaves to insure against systemic infection in order that a given plant might be used to supply leaves for several experiments. Another set of detached leaves was wiped with a synthetic mixture of common-mosaic virus and type A yellow-mosaic virus in the proportion of 999 parts to 1, and it was found that the yellow-mosaic virus did induce necrotic lesions.

When plants were known to be satisfactory for test purposes, 4 leaves were removed, 3 from *Nicotiana affinis* and 1 from *N. sylvestris*, and each was wiped with a virus obtained from a different tobacco plant having common mosaic. No necrotic lesions developed on these leaves. Each leaf was used to inoculate healthy tobacco plants. In all, 18 plants were inoculated. All plants developed common mosaic, and the delayed expression of from 1 to 4 small yellow-mosaic spots

occurred on each plant. Three of these spots were tested on *N. sylvestris* and the viruses were found to induce necrotic lesions.

Another test was carried out with a plant of *Nicotiana sylvestris* known to be a positive tester for the lesions of type A yellow mosaic when in combination with the virus of common mosaic. One leaf of this plant was trimmed, wiped with extracts of common mosaic and of yellow mosaic, and rinsed with water. The method is illustrated in figure 4. Care was exercised to confine the virus extracts to the areas wiped. The leaf was left attached to the plant. Necrotic lesions appeared on the margin wiped with virus of yellow mosaic. No necrotic lesions appeared on the half of the leaf wiped with virus of common mosaic, but yellowish-green areas did appear and common mosaic finally developed in the new leaves. As soon as mosaic appeared in the young leaves the inoculated leaf was removed. The portion of this leaf lying between the midrib and near the border of the necrotic lesions was cut into two strips, sections *b* and *c* as shown in figure 4, and each strip was pulped in a small amount of water. The resulting inoculum from each strip was wiped separately on attached leaves of two plants of *N. sylvestris*. All these leaves remained free of lesions. Common mosaic appeared on the new leaves of the plant inoculated with extract from the strip nearest to the midrib, but the plant inoculated with extract from the other strip remained healthy. A detached leaf from each of these plants was wiped with virus of type A yellow mosaic and typical lesions appeared, showing that the plants were of suitable test value.

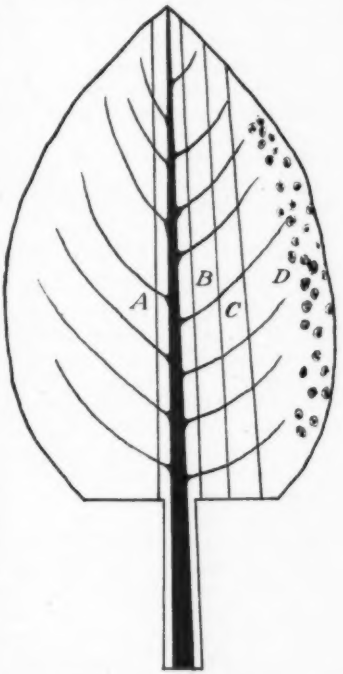

FIGURE 4.—Diagram of a leaf of *Nicotiana sylvestris* illustrating a method of inoculation used to determine whether the virus of type A yellow mosaic is detectable in the virus extract of common mosaic, and whether the virus of this yellow mosaic passed into tissues far removed from the lesions. The leaf was left attached to a healthy plant and inoculated in the following manner: The base was trimmed as shown, section *a* was then wiped with a virus extract of common mosaic and section *d* was wiped on the margin with virus extract of yellow mosaic. No necrotic lesions developed in section *a*, but many lesions developed on the margin of section *d*. When common mosaic appeared in the small central leaves of the plant the inoculated leaf was removed. Sections *b* and *c* were cut from the leaf and each was pulped separately, and the resulting extracts were wiped on leaves of separate plants of *N. sylvestris*. No necrotic lesions appeared on these leaves, but common mosaic appeared in the plant which was wiped with the extract from section *b*. The plant wiped with extract from section *c* remained mosaic-free.

These results seem to support the view that the virus of type A yellow mosaic did not pass from the leaf illustrated in figure 4 into the new leaves. The small central leaves showing common mosaic were removed and pulped. The resulting extract was wiped on leaves of *Nicotiana sylvestris* and also inoculated into tobacco plants. No necrotic lesions developed on the leaves of *N. sylvestris* but common mosaic did develop. Only the green mottling of common

mosaic appeared on the plants during the first 4 weeks of growth, but after that a few small yellow-mosaic spots appeared on each plant.

Virus extracts were obtained from some of these yellow-mosaic spots and wiped on the leaves of *N. sylvestris*. In some cases only the typical necrotic lesions developed and in other cases a systemic type of yellow mosaic resulted. The systemic type of yellow mosaic was not in evidence in previous tests of the stock virus of type A yellow mosaic.

The results obtained in the tests with *N. sylvestris* and *N. affinis* seem to warrant the conclusion that the yellow-mosaic spots which appeared in the test tobacco plants resulted from viruses which originated in the tissues involved in the spots and not from virus which had been carried along as a contaminant with the virus of common mosaic. As in all other methods used, this method supports the view that the virus of common mosaic mutates.

TYPES OF YELLOW MOSAIC ISOLATED FROM TOBACCO

It was pointed out in an abstract (*17*) that several types of yellow mosaic have been isolated. Three of these are described briefly here.

Type A.—This yellow mosaic resembles Johnson's (*9*) virus no. 6. It was isolated by the writer from the common mosaic which was used throughout the present studies. At high summer temperatures tobacco plants develop a bright yellow or sometimes a creamy white mottling. When growing temperatures are reduced the yellow color of the mottled areas of subsequent foliage gives way to a light green as a result of a gradual increase in the chlorophyll content. At temperatures of about 13° to 15° C., this yellow mosaic is indistinguishable from the common mosaic on tobacco plants grown at temperatures of about 21° to 24°, yet the virus from plants grown at these low temperatures induces typical yellow mosaic when inoculated into plants cultured at 21° to 24°, thus indicating that the symptom changes are not due to a mixed virus.

On the Canary Island strain of *N. glauca*, symptoms are either absent or very mild, especially at 21° C. and below, and the virus is not carried in mosaic-free leaves and stems in the upper portion of plants that are allowed to continue growth for several weeks or months after inoculation.

The virus produces necrotic lesions on the leaves of *N. rustica*, *N. glutinosa*, *N. sylvestris*, and certain strains of *N. affinis* at 21° to 24° C. The properties of the virus in vitro are similar to those of the virus of common mosaic.

If the strain of *N. glauca* used by Johnson (*9*) is genetically the same as the writer's strain, then type A yellow mosaic may be the same as Johnson's no. 6 yellow mosaic, for he states that the symptoms are often masked on *N. glauca*. Jensen (*8*) has described a yellow mosaic (isolation no. 102) that shows symptoms like the writer's type A yellow mosaic on tobacco, and he considers his mosaic to be similar to Johnson's no. 6 and to aucuba mosaic, but, according to Kunkel (*11*) aucuba mosaic induces necrotic lesions on leaves of *N. sylvestris*, whereas Jensen's does not.

Type B.—This yellow mosaic was isolated from a yellow spot associated with a green mosaic on tobacco. The original virus was in *Nicotiana glauca* collected on the island of Grand Canary. The

mild dark-green mosaic referred to in this paper predominated in this material and a common mosaic was also isolated with the yellow spot. At high summer temperatures this mosaic on tobacco has been indistinguishable from type A. However, chlorophyll masks the yellow areas of type B mosaic more rapidly at lower temperatures than it does those of type A. At temperatures near 15° to 19° C. the symptoms of type B mosaic on tobacco resemble those of common mosaic on tobacco when grown near 21° to 24° C.

On the Canary Island strain of *N. glauca* this mosaic is characterized by very severe yellow mottling on all leaves, stems, and petioles, and the virus is systemic in all these parts.

The virus produces necrotic lesions on the leaves of *N. rustica*, *N. glutinosa*, *N. sylvestris*, and certain strains of *N. affinis* at 21° to 24° C. The lesions resemble those induced by the virus of type A. The properties of the virus in vitro are similar to those of the virus of common mosaic.

If the strain of *N. glauca* used by Johnson is genetically the same as the writer's strain, then type B yellow mosaic appears to differ from Johnson's no. 6 yellow mosaic, as the writer has always obtained severe symptoms at temperatures above 21° C. on this host. Strain differences in this host must be reckoned with in virus studies, as the writer's tests indicate that mottling is more severe on the Canary Island strain than on the Babcock and Clausen strains.

Type C.—This yellow mosaic was isolated from a yellow spot associated with a green mosaic resembling common mosaic on tobacco. The original virus was in *Nicotiana glauca* collected by the writer on the island of Grand Canary. The yellow pattern of this mosaic on tobacco is distinct from that caused by types A and B. The designs take on the appearance of lace and the degree of yellowing is less than in types A and B. The virus of this mosaic does not produce symptoms on tomato, and tomato is not a carrier of the virus. On the Canary Island strain of *N. glauca* the symptoms appear on all leaves, but they are less severe than those induced by type B. The virus produces necrotic lesions on the leaves of *N. rustica*, *N. glutinosa*, and *N. sylvestris* at 21° to 24° C. The virus retains its potency in dry tissue for at least 29 months at room temperatures. The thermal destruction point of the virus has been between 80° and 85° C. in several tests.

During the process of purifying this virus it was found that a mild dark-green mosaic was associated with it. This green mosaic is very similar to, if not identical with, the mild dark-green mosaic used in the experiments cited in this paper. Since the virus of type C yellow mosaic possesses several properties in common with the virus of mild dark-green mosaic, it is suspected that this yellow-mosaic virus may have originated from the virus of mild dark-green mosaic under special conditions not yet maintained in the writer's experiments.

DISCUSSION

It has been shown that a few small yellow spots occurred in all the collections of common mosaic studied. After the isolation and purification of the viruses from these spots it was found throughout long series of subinoculations that the viruses induce symptoms which are a distinct departure from those of common mosaic.

Tests indicate that these viruses arise spontaneously in association with common mosaic and not from contaminations from outside sources and the evidence presented seems to support the view that these new viruses represent mutants and not loosely fixed variants.

The precise mechanism involved in the spontaneous origin of one virus from another is not known. Assuming the virus to be living and capable of independent regeneration, the process may be similar to the saltation or mutation in fungi and in higher organisms. However, it seems likely that the virus is no larger than, if as large as, some genes, and therefore the mutation would involve a very minute physical-chemical mechanism as in the case of genes. It is possible that the process involves changes in a chemical compound due to the addition of or the dropping of a radical, to alterations in valence, or to isomeric changes. If the plant cells provide a special mechanism for increasing the virus, it is possible that the first step in the mutation of the virus consists of a change in the function of the virus-producing mechanism.

The fact that yellow-mosaic spots appeared on all of the many plants with common mosaic might be advanced as an argument against the mutation of the virus on the ground that the occurrence is too frequent. However, this argument carries little weight for the reason that the frequency must be based on the total number of virus particles involved in all of the plants and not on the number of plants. On this basis the millions of virus particles involved in a single plant would indicate a low frequency at the temperatures employed in these tests.

The expression of the yellow-mosaic spots seems to be influenced by the environment; therefore this factor must be reckoned with in studies on this problem. It is entirely possible that some of the viruses and hosts found suitable for these studies might not be suitable under environmental conditions differing markedly from those maintained in this work. Host material which is suitable for developing evidence on the virus-mutation theory with certain yellow mosaics and common mosaics of tobacco may be unsuitable for another combination. For example, *Nicotiana glauca* was satisfactory for type A yellow mosaic and the common mosaic associated with it, but not for type B yellow mosaic and the common mosaic associated with it because both of these latter viruses induced symptoms and were systemic in *N. glauca*. Heterozygosity in test plants must be kept in mind constantly in virus-mutation and classification studies. This was especially evident in the studies involving *N. affinis*. In critical work where a considerable number of plants are needed it is sometimes necessary to propagate vegetatively from single plants in order to reduce genetic irregularity to a minimum.

In addition to the 3 yellow mosaics isolated in these studies, Jensen (*8*) isolated 3 yellow mosaics from common mosaic of tobacco, and Price (*23*) isolated several yellow mosaics from cucumber mosaic. In wheat the writer (*18*) reported the isolation of a yellow mosaic which is closely associated with a green mosaic, and later he [7] reported another yellow mosaic which is closely associated with another green mosaic. Since that time another similar association

[7] McKinney, H. H. Wheat mosaic in Kansas. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rept. 16: 115-116. 1932. [Mimeographed.]

has been found. These yellow mosaics in wheat may originate in the same manner as those in tobacco, but the evidence is less readily obtained. However, in the case of the green-mosaic-rosette virus repeated isolations from green mottled tissues for 5 years have failed to eliminate all traces of the yellow mosaic, leaving one to infer that the mutable characteristic is common to the green-mosaic rosette of wheat.

A foliage-deforming green mosaic has been isolated from a light-green spot associated with the mild dark-green mosaic used in these studies.

In addition to the severe yellow mosaics which arise from common mosaic, it appears from the results presented by Holmes (7) that a certain masked mosaic arises from a mosaic which induces mottling.

Virus mutation opens up an interesting and important field dealing with the origin of new types, the evolutionary development of viruses, and their relationships. The new types obtained thus far from established types appear to possess many properties in common with the virus from which they are considered to have originated. The most striking departure of a new type thus far has been in the relative intensity of the symptoms induced in the host, accompanied by relatively minor changes in host range and other properties. Minor changes in properties may signify only strain differences, but regardless of this point there seems to be ample justification for considering that another virus has been isolated even though it may show a consistent departure from the original in but one property or characteristic. As this field of study is opened up it seems entirely possible that viruses may be obtained which will show even more extreme departures from the originals. A virus manifesting a radical departure might arise through a single mutation, but it seems more likely that extreme changes in properties and in host range are eventually effected as mutation proceeds through several stages involving different varieties and species of plants growing in different environments.

The virus of tobacco common mosaic and the virus of cucumber common mosaic may be thought of as representing two distinct central or basic types, each fixed within limits but capable of producing by mutation its particular types or strains which in turn may mutate. While the writer is inclined to the view that the mutants from these two viruses will probably fall for the most part into two distinct groups or ranges, it is possible that some of the mutants in the two groups may be very similar to each other or even overlap in certain characteristics. From the studies on the tobacco common mosaic the writer is inclined to the view that this virus is not fixed to the extent that most workers believe. It is strongly suspected that some of the collections of common mosaic show differences of a magnitude which are fully as great as the differences exhibited between some of the yellow mosaics that arise as mutants from common mosaic. It seems entirely possible that the virus of tobacco common mosaic may have originated from another virus which may or may not induce mottling symptoms in tobacco. It is not beyond the realm of possibility that there may be viruses which cannot induce any of the now recognized symptoms in any known species. However, mutants from such viruses might be capable of inducing signs of disease.

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It seems probable that several strain types of virus resulting from mutation are present in many tobacco plants having common mosaic, and that this may occur in many virus diseases. Such a complex may explain the so-called attenuation and the increased virulence attributed to the exposure of certain viruses to extreme temperatures, passages of virus through different plants or animals, and to other treatments.

The role of mutation in epidemiology has several angles which should be considered. Throughout the studies at the Arlington farm, the pure yellow mosaics of tobacco have been handled under quarantine, and all diseased plants and the soil in which they grew have been sterilized with steam before being disposed of. On tobacco and tomato the yellow mosaics are much more destructive than green mosaic, and with the exception of the green-mosaic rosette on Harvest Queen wheat and similar varieties, this is true among susceptible varieties of wheat. On the other hand, there are certain natural factors which tend to restrict certain of the yellow mosaics.

It was pointed out in an earlier paper (*15*) that pure yellow mosaics occur less generally than green mosaic on *Nicotiana glauca* in the Canary Islands. The same was found to be true in commercial fields of tobacco visited in Pennsylvania, Virginia, and Maryland. This is explained on the basis that there are few sources of pure yellow mosaic to serve as centers for spread. These are few because of the extremely small possibility of transmitting pure yellow mosaic from the relatively small number of yellow spots that appear on plants having common mosaic. Measurements made on a typical tobacco plant with common mosaic showed that the total leaf area was approximately 13,600 cm^2, and in this area about 1 cm^2 was involved in yellow-mosaic spots. From this it is clear that natural and cultural factors could rarely be expected to successfully establish pure yellow mosaic in another plant from the small spots. On the other hand, the chances are essentially reversed when plants with pure yellow mosaic are exposed to natural factors in a field.

The characteristic action of common-mosaic virus in gradually suppressing or inhibiting at least certain yellow-mosaic viruses arising from it doubtless plays an important role in delimiting the size of the yellow-mosaic spots. This characteristic also militates against the rapid establishment of cases of pure yellow mosaic under natural conditions. However, if these yellow mosaics get into new areas or on new varieties where the suppressing influence may be reduced or possibly reversed, they are likely to be very destructive.

The suppressing influence of one virus over another is not fully understood, but it may be that the phenomenon represents differences in the ability of the viruses to compete in their development, a situation which may exist without reference to a special defensive mechanism set up by the plant. It seems entirely possible that this phenomenon is similar to the antagonism between certain fungi and between certain bacteria.

Kunkel (*11*) in his study of the yellow-mosaic and common-mosaic viruses on *Nicotiana sylvestris* concluded that the virus of common mosaic induces an acquired immunity in the plant against the virus of yellow mosaic. In similar studies Caldwell (*3*) referred to the phenomenon as induced immunity. The general conception of ac-

quired immunity has grown up to a very large extent around the classic example of immunization against smallpox by a presumed special defense mechanism set up in the body as a result of the immunizing agent. However, a review of the literature on acquired immunity in mammals shows that there are a number of types which differ to a considerable extent from the situation obtaining in acquired immunity to smallpox, and that acquired immunity is relative to a considerable degree.

The writer's tests have shown that the common mosaic gradually suppresses the yellow-mosaic virus in the meristematic tissues, thereby effecting what possibly may be considered a cure for yellow mosaic. Leaves already displaying yellow mosaic continued to show it until they died, but before the end of the growth cycle of the plant the yellow mosaic was to all intents and purposes cured in the leaves that were produced after those in which the blended symptoms appeared. Furthermore, common mosaic developed on side branches from the axils of leaves which had expressed yellow mosaic before the reinoculation with virus of common mosaic. Preliminary studies have indicated that the suppression of the yellow-mosaic symptoms is more rapid than the suppression of the virus. In the early stages of recovery from yellow mosaic the yellow-mosaic virus is present in considerable amount in leaves showing only the light- and dark-green mottling of common mosaic.

This so-called "curative characteristic" is not an outstanding attribute of the agents which immunize against virus diseases in mammals, though in the case of rabies the disease is prevented when the Pasteur treatments are administered before the completion of the incubation period. Alleviating effects have been claimed for some of the antitoxins and for the antiserum used against cerebrospinal meningitis.

It is true that tobacco plants inoculated with the virus of common mosaic are protected against the occurrence of pure yellow mosaic, but the symptoms of common mosaic remain and the disease persists throughout the life of the plant when grown under normal culture conditions. This characteristic is distinctly at variance with the situation found in acquired immunity among mammals.

If we hold strictly to the prevailing interpretation of acquired immunity as exemplified in mammals, it is difficult to consider the mosaic relationship in question as representing bona fide acquired immunity. On the other hand, it is quite possible that the prevailing interpretation is too limited for the purpose of the plant pathologist, and that the virus of common mosaic should be looked upon as an immunizing agent or "vaccine" which protects the tobacco plant against the yellow mosaic. It seems unnecessary to assume that the virus of common mosaic induces the plant to set up a special protective mechanism, such as a system of antibodies. It appears also that the common-mosaic virus represents a rather low form of "vaccine" since the disease produced by it is permanent. On the other hand, the virus represents a relatively high form of "vaccine" since ultimately it renders a "cure." The *G* virus used in Salaman's (*24*) tests against the *L* virus in tobacco and *Datura stramonium* is a more efficient "vaccine" since it induces very slight symptoms with no appreciable effect on the health of the plants. Simon (*25*) has pointed out in connection with the control of smallpox that during the early period

when prophylaxis was obtained through variolation certain people so treated served as carriers of the smallpox virus though they were immunized against the disease. This danger was not removed until the cowpox vaccine method was used. In the case of tick fever, cattle which have completely recovered from an attack of the disease carry the parasite in the blood for long periods.

In view of the mutable nature of some of the plant viruses, it is possible that in time viruses may be isolated which will protect as well as "cure" and yet will not survive indefinitely in an active form in the plant.

SUMMARY

Twenty-three collections of viruses which induce common mosaic or similar types of green mosaic on tobacco were obtained from different parts of the world. All of these mosaics developed a few bright yellow or yellowish-green spots of small size on the foliage of each tobacco plant tested. Three other viruses which induce green mosaics distinctly not of the common type on tobacco did not induce these yellow-mosaic spots.

These spots contain viruses which are distinct from the virus of common mosaic.

After the isolation and purification of viruses from these spots it was found through a long series of subinoculations that new symptoms are consistently associated with the new viruses. Thus the new viruses represent permanent departures from the established type, the essential criterion for mutation.

The yellow-mosaic viruses from the common mosaic do not compete successfully with the virus of common mosaic in tobacco. When both viruses are introduced into the plant in approximately equal parts yellow mosaic develops, but the virus of common mosaic gradually predominates until the syptoms of common mosaic take possession of the top leaves. The same results obtain when plants with yellow mosaic are reinoculated with the virus of common mosaic, but when plants with common mosaic are reinoculated with the virus of yellow mosaic no changes are apparent in the symptoms.

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ROOT DEVELOPMENT OF PITCH PINE, WITH SOME COMPARATIVE OBSERVATIONS ON SHORTLEAF PINE [1]

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INTRODUCTION

The development of the root systems of trees constitutes a relatively unexplored field of botany. Most of the older references to this subject have been based on casual observations of exposed roots along roadside cuts and eroded stream banks, or of the upturned roots of wind-thrown trees. Hence, descriptions of the root systems of trees generally have been incomplete, if not actually inaccurate.

The extensive investigations of the roots of herbaceous and shrubby plants, particularly those by Cannon (*5*) [4] and Weaver (*26*, *27*, *28*), led naturally to a greater interest in the roots of trees. However, the relatively large amounts of time and labor required have tended to discourage work in this field. Problems arising in the nurseries and the relative facility with which small plants can be studied have directed research mostly toward the seedling stages. Toumey described and classified the seedling root systems of many of the more important eastern species (*25*) and stimulated many other investigations of a similar character.

A few studies of the roots of older trees have been carried out in this country. In general, the work done thus far has been of a preliminary character, and gives neither a complete picture of the root growth of a species from seedling to maturity on any particular site nor an adequate account of the root reactions of a species to the various sites on which it may grow. Woodroof's studies (*30*, *31*) on the pecan constitute an exception to this statement.

Somewhat more work has been done in the north-European countries. The German work has been summarized by Büsgen and Münch (*4*). Perhaps the most complete study of any one species was carried out by Laitakari (*17*) in Finland on Scotch pine (*Pinus sylvestris* L.).

The species chosen for the present investigation was pitch pine (*Pinus rigida* Mill.), because of its wide distribution throughout the Eastern States and its remarkable tolerance of a wide range of unfavorable situations. Particularly because of this latter quality, the species promises to assume increasing importance in the forestation programs of the future, although it is not an important timber tree at present. Pitch pine is prominent among the botanically interesting flora of the pine barrens of New Jersey. The ability of the species to withstand fire evokes the wonder and admiration of all who know that unique section.

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[3] Maintained at Philadelphia, Pa., in cooperation with the University of Pennsylvania.

[4] Reference is made by number (italic) to Literature Cited, p. 1015.

Definite root studies of pitch pine have apparently never been made. Harshberger (*10*) made some general statements concerning the root development of the species, and published a small drawing of a tree with its root system. His observations obviously were of a most cursory character, and his figure is entirely inadequate as a portrayal of a root system. Illick and Aughanbaugh (*15*) mention the strong taproot of pitch pine seedlings and the fact that this root becomes relatively less significant in older trees. They measured the upturned root mass of a wind-fallen tree, recording 18 feet as the diameter of the root system of a specimen 82 feet tall. Such figures are of questionable value; probably not more than one-fourth of the actual diameter of that root system was represented in the mass torn from the earth when the tree fell.

The observations of Harshberger and of Illick and Aughanbaugh are representative of the available information on the roots of pitch pine, and of most other tree species. More precise knowledge of the root habits of trees is requisite for the judicious planning of forestation programs. In this investigation, the major objective was to study, on one site, a developmental series of root systems of pitch pine from the seedling stages to maturity. Secondary objectives included comparative observations of the root systems of normal and weak trees, of trees growing on different sites, and of pitch pine and shortleaf pine (*Pinus echinata* Mill.) growing on the same site.

FIELD WORK

DESCRIPTION OF SITE

The major part of the field work was done on the tract of the Allegheny Forest Experiment Station in the Lebanon State Forest in New Jersey. Some data were obtained from the Ockanickon area of the experiment station near Medford, N. J., and from the Mont Alto State Forest in southern Pennsylvania. Both of the locations in New Jersey are in the pine barrens, the ecology of which has been treated in some detail by Harshberger (*10*).

The site of most of the excavations on the Lebanon forest was an area of about 40 acres which, being traversed by a low ridge, varies from level to gently sloping. Drainage is good; excavations as deep as 9 feet did not encounter water, even on the lower ground. The soil of the ridge is typical Lakewood sand; on the lower ground it tends somewhat toward the Sassafras types. At a few places, a slightly harder, dark-colored layer was encountered below the A horizon, suggestive of the St. Johns series, but it was not well developed. These minor variations in soil did not seem to exert any appreciable direct effect on the behavior of the pine roots.

A recent paper by Lutz (*20*) gives detailed data on the soils of the section and a quantitative expression of their poor quality. His determinations show that the pine-barren soils are characterized by: (1) High percentage of sand; (2) low percentages of clay and total colloids; (3) low content of organic matter; (4) low contents of nitrogen and phosphorus; (5) high carbon-nitrogen ratio; (6) high acidity; (7) excessive leaching, and varying degrees of podsolization; (8) low water-holding capacity; and (9) periodic occurrences of low percentages of available moisture—the moisture content may fall below the wilting coefficient during periods of drought.

The forest cover is a mixture of white, black, and chestnut oaks (*Quercus alba* L., *Q. velutina* Lam., *Q. prinus* L.) with pitch and shortleaf pines. The oaks are mostly sprout growth averaging 4 to 5 inches in diameter breast high,[5] and represent the growth since the last cutting for charcoal. The pines are of all sizes up to 12 inches d. b. h. Some of the larger ones are approaching, or perhaps exceed, 100 years of age. Apparently the area has not suffered severe burning for some time, as seedling and sapling pines of various sizes are fairly abundant.

The ericaceous ground cover, typical of the section, is sparse on the ridge, and composed mostly of dry-land blueberry (*Vaccinium vacillans* Kalm). Lower down on the slopes, black huckleberry (*Gaylussacia baccata* (Wang.) C. Koch) becomes predominant, with dangleberry (*G. frondosa* (L.) Torrey and Gray) appearing also on the lowest flats. A dense ground cover of the huckleberries rather effectively excludes pine seedlings. The natural inference is that saplings and older trees growing among them got started following a ground fire which had temporarily checked the huckleberries. The majority of the pines in seedling stages are now found on the higher and less densely vegetated parts of the area.

METHODS

The root systems were exposed by the dry method. The first step in the case of trees larger than sapling size was to uncover the root crown to a depth of 1 foot or more and radially about 3 feet. This operation was, of necessity, largely done by hand in "badger" fashion. With the root crown thus revealed, the most suitable position for making deeper excavations with the least destruction of roots could be determined. A hole of sufficient size to allow a man to stand in it and use a spade was then dug alongside the stump. Its ultimate depth and the size necessary to permit spading movements and prevent caving of the walls were determined, of course, by the depth of the taproot. By careful caving of the wall toward the stump, the root crown and taproot could be clearly exposed without serious mutilation. With the bases of the lateral roots thus revealed, representative ones could be selected and followed in any degree of detail desired. Exposure of the laterals also was largely a hand process, although a trowel and small hand ax were useful. An ice pick was helpful in freeing the finer roots from the soil. Excavation of vertical branches of the large laterals necessitated digging a hole in the same manner as for the taproot.

The dry method has several advantages over the alternative wet method, in which the roots are washed from the soil with a stream of water under pressure. The former can be used by an investigator working alone, it does not require proximity to a source of water or to a road by which water may be hauled, and it requires only the simplest outlay of equipment. A spade, an ax, a trowel, a hand ax, an ice pick, a ruler, and a few small pails or jars of water for saving specimen roots constitute the essentials for field work. To get complete detail of the finer root branches in sandy soils by dry excavation is not unduly difficult. The wet method is more suitable, however, for getting out seedling root systems, or parts of larger

[5] Four and a half feet above the ground; abbreviated "d. b. h."

systems, intact. Considerable breakage and desiccation of finer roots are unavoidable by the dry method.

In this study, no attempt was made to obtain complete intact root systems except in the case of small seedlings. On larger trees the taproot and only a limited number of representative primary laterals were followed to the tips. Likewise, only representative samples of the higher orders of branching were examined in detail. Experience soon showed the general range of variation to be expected. Excavation of all the roots would have consumed more time than the results would warrant in a study of general root habit.

GENERAL FEATURES OF PITCH PINE ROOT SYSTEMS

The root system of pitch pine may be regarded as belonging to the generalized type, i. e., it is both widely spreading and deeply penetrating, but does not attain extreme development in either direction.

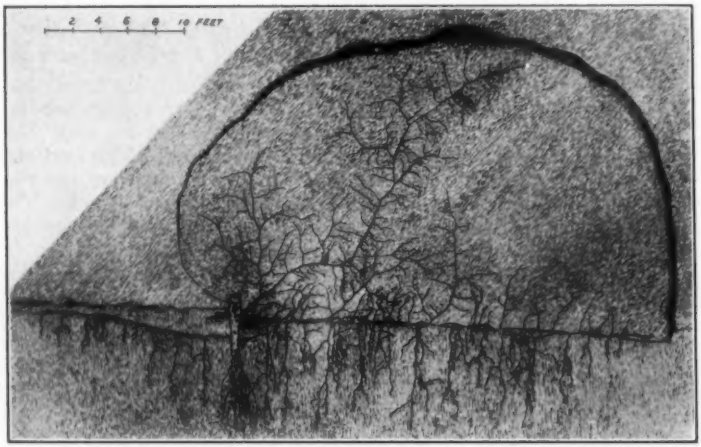

FIGURE 1.—Three-dimensional diagram illustrating the taproot and the positions of three primary laterals with their larger secondary and tertiary branches. The development of vertical roots is depicted at the right; at the left is shown a lateral of deeper origin obliquing upward; in the background is shown the horizontal development with the topsoil removed. Modeled after a tree about 30 years old.

Insofar as we have knowledge of other pines, this type of root system seems to be typical of the genus (*6, 12, 17*). It usually shows a definite and fairly strong taproot which frequently is found to divide, below a depth of 2 to 3 feet, into numerous branches descending at acute angles to the vertical. This feature becomes apparent only after the tree has passed the sapling stages. The major part of the lateral system originates from the root crown within 8 inches from the soil surface. These primary laterals extend radially at depths of 2 to 8 inches, rarely, if ever, turning downward appreciably. Where a dense mat of ericaceous roots and rhizomes occurs, the pine roots tend to run below it at depths of 5 to 8 inches; where the ericaceous mat is absent or poorly developed, some pine laterals will be found as close as 2 inches to the soil surface. The primary lateral system is almost entirely confined to the surface soil during the seedling and

sapling stages; later, laterals originating as deep as 2 feet may attain conspicuous size. Deeply originating laterals frequently, though not always, oblique gradually upward to the surface soil, where they proceed horizontally. The primary laterals give off both vertical and horizontal secondary branches, which rebranch commonly to the fifth and sixth orders. The term "vertical", as here used, applies only to roots growing downward; those branches which grow upward from the more deeply situated laterals soon turn in a horizontal direction and are not distinguished from those which grow horizontally from the start. The vertical branches along the basal half of a lateral may penetrate almost as deeply as does the taproot; those of more recent origin on the distal half of the lateral naturally are not so well developed (fig. 1). That the tree has contact with the subsoil under the major part of the area covered by the lateral system is a fact that is not generally appreciated and that warrants emphasis.

Many of the ultimate branches are mycorrhizal. Lateral branches of the higher orders frequently extend into the duff, where mycorrhizal development may be profuse. However, mycorrhizae are not confined to the surface soil. In the pine-barren soils, mycorrhizae seem able to develop under practically all conditions that permit growth of the pine root itself. They were observed as deep as 8 feet in drained soils and more than 3 feet below the water table in saturated soils.

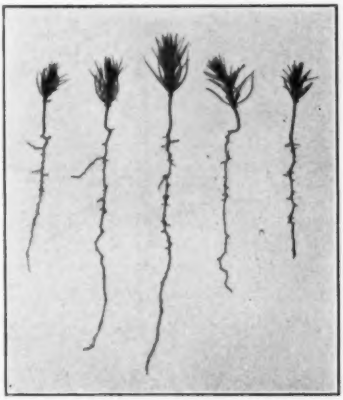

FIGURE 2.—Specimens of *Pinus rigida* seedlings taken in June from open woods. The length of the longest taproot was 5.5 inches.

ROOT DEVELOPMENT IN EARLIER STAGES OF GROWTH

SEEDLING ROOT SYSTEMS

The taproot is the most conspicuous feature of seedling root systems. Taproots of seedlings in their first year of growth varied in length from 3 inches to more than 1 foot (fig. 2). The influence on root development of local differences in site is probably more clearly seen in young seedlings than at any later stage. The maximum elongation of roots occurs on areas of clean, loose sand subjected to strong insolation. The bare sand of infrequently used roadways or trails will often yield seedlings with exceptional root development. The combined factors of shade, higher humus content of the soil, and underground competition tend to retard root elongation of seedlings growing under the tree canopy. Under these conditions, the taproots sometimes penetrate scarcely deeper than through the layers of duff and raw humus during their first year of growth. Burns (*3*) has shown experimentally that white pine seedlings produce the strongest root systems when grown without shade, the root system

becoming progressively weaker as shade is increased. Top development, also, was markedly retarded in the plants grown in full shade. In the relatively open woods of the pine barrens, conditions of full shade rarely occur. Hence, top development gives little indication of root development in the case of young pitch pine seedlings in nature.

By the end of the first year, recognizable lateral roots have appeared along the upper 2 inches of taproot (fig. 3). Although the strongest 2 or 3 laterals on well-developed root systems may be as long as 6 inches, most of them do not exceed 3 inches in length. Usually not more than 5 or 6 branches can definitely be identified as potential laterals on the 1-year-old seedling. At this time branching has proceeded to the second and occasionally the third order.

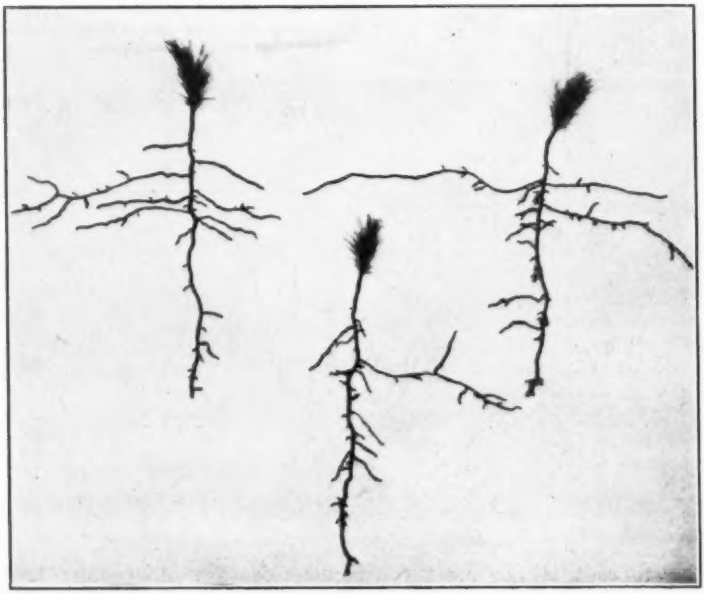

FIGURE 3.—Specimens of *Pinus rigida* seedlings taken in August from an exposed roadside. The taproots were about 8 inches long.

Mycorrhizae were already evident in June on the roots of seedlings of the current season. Such seedlings could not have been much more than 2 months old. Some apparently healthy seedlings were found on which the whole root system was sheathed in fungus mycelium. Mycorrhizae were present in abundance on 1-year-old seedlings, with some clusters showing 4 or 5 dichotomies.

The taproot may be regarded as dominating the root system up to the eighth or tenth year of the plant's life, though after the first year its dominance steadily decreases. Seedlings 4 or 5 years old, growing under conditions of moderate competition and partial shade on Lakewood sand, showed taproot penetrations of 15 to 24 inches and maximum lateral extensions of 12 to 15 inches (fig. 4). Usually there

were 6 to 8 horizontal roots exceeding 6 inches in length, the largest of which approximated the size of the taproot. Even at this early stage, the increasing importance of the lateral system is becoming apparent.

Seedlings of this age (4 or 5 years old) are typically 10 to 12 inches tall, unbranched weak, and spindling, decumbent or, at best, only feebly erect. The seedlings of both *Pinus rigida* and *P. echinata* usually topple over during the second or third year, and remain in a semiprostrate position for several seasons. By the eighth year they usually have again assumed an erect position, but a permanent crook remains at the ground line. This crook is ultimately obscured by the thickening of the stem, but frequently can still be seen in saplings 8 or 10 feet tall.

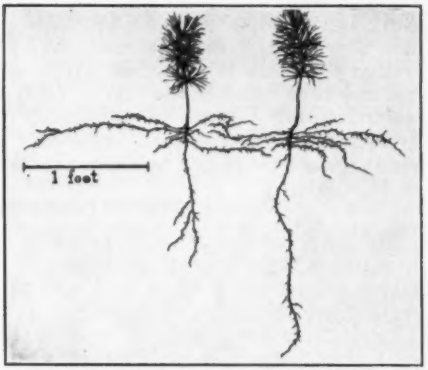

FIGURE 4.—Four-year-old seedlings of *Pinus rigida* with practically complete root systems.

Seedlings 8 to 9 years old, likewise under conditions of moderate competition and partial shade, were 1.5 to 2.5 feet tall and were beginning to branch. Though plants of this age have assumed a fairly erect position, the stems still lack rigidity. Naturally, varia-

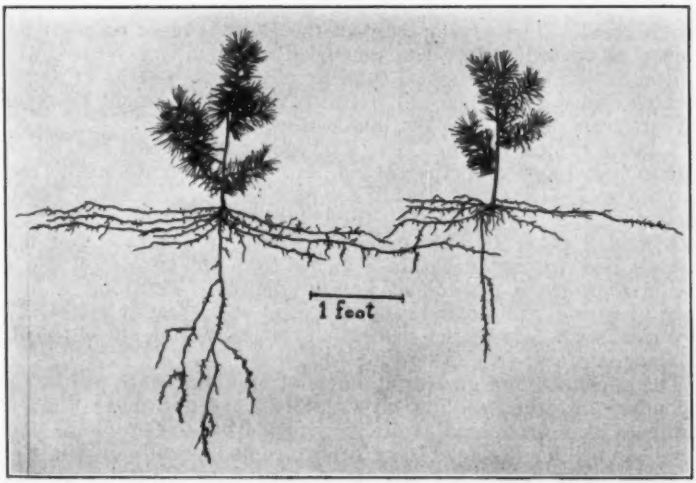

FIGURE 5.—Eight-year-old seedlings of *Pinus rigida* with practically complete root systems.

tion between individuals increases with age, both above and below ground. Taproot penetrations varying from 21 to 34 inches were found on sites apparently very similar (fig. 5). The stronger laterals approximated 2 feet in length, though one was found which measured

40 inches. The number of identifiable laterals averaged 10 or more. Some of the stronger ones bore secondary branches almost 1 foot long. Branching had proceeded commonly to the third and occasionally to the fourth order, exclusive of mycorrhizae.

Some comparison was made with seedlings of *Pinus echinata* of the same age and on the same sites. The development of the two species was very similar; no differences were noted which did not readily fall within the limits of variation within each species. It is the writer's opinion that detailed analysis of many specimens will be necessary to determine any differences which may exist between the seedling root systems of the two species. However, it was noted that the stems of seedlings of *P. echinata* tend to stand more rigidly erect than do those of *P. rigida*.

ROOT SYSTEMS OF SAPLINGS AND SMALL TREES

By the eleventh or twelfth year the pitch pine seedling has acquired a degree of rigidity heretofore lacking, the growth rate has increased, giving an aspect of vigor, and annual whorls of branches are being regularly developed. The young plant at this age begins to appear like a tree. This acceleration of growth undoubtedly is coordinated with root development; it could not take place until an adequate absorbing system had been formed and sufficient contact made with the subsoil to insure a constant water supply. The dimensions attained by the roots indicate an even more striking acceleration of growth underground than that displayed by the tops. The eighth to tenth years mark the inception of a period of rapid root elongation. This period also witnesses the appearance of secondary branches of conspicuous size.

It should be borne in mind that, although it is convenient to speak of these younger stages in terms of years, age probably is relatively insignificant. The development of the plant is more properly to be viewed as an orderly process, the speed of which is governed, within limits, by the environmental conditions. Thus, a stage of development ascribed to 10-year-old trees in New Jersey might be reached several years earlier, or later, under other conditions.

A fairly typical sapling, 11 or 12 years of age, was 4 feet tall, and 0.75 inch in diameter at the ground line. The current season's leader was 8.5 inches long, and that of the past season, 7 inches. The taproot reached a depth of 4 feet, the longest lateral root reached out 8 feet, and 3 other laterals exceeded 4 feet in length. Ten major laterals had originated within 6 inches from the surface of the soil, and 5 more were present at greater depths. The longest lateral bore 12 secondary branches ranging from 1 to 2.5 feet in length. The strongest secondary branch descended vertically from one of the other primary laterals to a depth of 3.5 feet.

The appearance of greater numbers of branches, both primary and secondary, as the seedling grows older does not mean that new branches have originated at points remote from the tip of the parent root. As will be discussed later, branch roots normally originate only in tip regions before the advent of secondary thickening. Many branches never become more than a few inches long, and eventually die and disintegrate. Certain ones, however, which at first are undistinguishable from the ephemerals, later undergo increased growth in both length and thickness and become recognizable as permanent structures. These account for the apparent increase in the number of major branches.

The root dimensions given indicate the relative decline of the taproot and the mounting prominence of the system of primary lateral branches. The taproot, of course, retains its importance as an organ of central anchorage, and as the mechanical and physiological center of the root system. Its upper part is the conduit through which all liquids must pass. Its function as an absorbing organ, however, becomes more and more insignificant as the tree develops. Vertical secondary branches, or sinkers, contact the subsoil and thus relieve the taproot of its earlier vital role—insurance against death by drought. Ultimately, the taproot, including its descending branches, may not greatly exceed the stronger sinkers with respect to the amount of absorbing area.

A somewhat larger sapling illustrates further the relative decline of the taproot. It also illustrates a fact borne out by observations on larger trees, that beyond the seedling stages the size of the top furnishes but little indication of the depth or size of the taproot. The lateral system, however, is fairly closely correlated with stem development. After a little experience, the observer can predict the general dimensions of the lateral system. This sapling was 14 years old by ring count, 7.5 feet tall, and 1.5 inches in diameter at the ground line. The taproot reached a depth of only 3 feet. It gave off 20 laterals, 14 of which originated at depths not greater than 6 inches. The longest one measured 10.5 feet, 3 others exceeded 8 feet in length, while the remainder ranged from 2 to 8 feet. Primary laterals shorter than 2 feet were not considered. The 2 strongest laterals each bore about 30 secondary branches approaching or exceeding 1 foot in length. Very few were more than 3 feet long. At least 3 vertical secondaries reached into the fourth foot of soil, thus exceeding the taproot in depth. Branching had proceeded to the sixth order, exclusive of mycorrhizae.

In the consideration of secondary branches, only those which approached or exceeded 1 foot in length were recorded numerically. Smaller branches were numerous, but irregularly distributed. Many were dead, or apparently dormant, and destined eventually to slough off. Their importance as absorbing organs is, at present, purely speculative, but would seem to be negligible on the older parts of the root system. A branch which has attained a length of 1 foot is growing, or previously has grown, more vigorously. Its basal parts have undergone some secondary thickening, and the epidermis has been replaced by the characteristic flaky bark, giving the aspect of a permanent organ. A reasonably close correlation exists between the basal diameters and the lengths of pine roots. Thus, their lengths can be estimated without complete exposure. Branching to the higher orders is considerably more profuse on lateral branches than on vertical ones.

Primary laterals less than 5 feet long rarely have many such secondary branches. The latter appear in increasing numbers as the primary elongates. By the time a primary has become 10 feet long, it may have 20 to 40 secondaries 1 to 4 feet long. Usually, one-half to three-fourths of these are horizontal, and the remaining one-fourth to one-half are vertical. Such a primary lateral thus has developed from a root into a root system in itself. Its zone of absorption extends down to the moister sands of the subsoil, and several feet laterally on either side in the topsoil. Subsequent growth of the pri-

mary lateral is simply the extension, vertically, laterally, and linearly, of this zone of root occupancy. However, downward growth does not keep equal pace with the lateral and linear advance. Hence, the zone of root occupancy of a primary lateral undergoes a gradual relative flattening as development progresses, and a constantly increasing proportion of the root system as a whole becomes localized in the surface soil. The data on larger trees illustrate this tendency.

A specimen tree, 14 feet tall, 2.5 inches d. b. h., and about 17 years old, may be cited. This individual had originated as a sprout, and therefore probably was slightly larger for its age than trees developing directly from seed. Suitable specimens of seedling origin of this general size were almost entirely lacking in the locality. The taproot was rather atypical. It divided about 9 inches below the surface of the soil, the two roots subsequently uniting in a natural graft, and then redividing. One branch ended at a depth of 3 feet. The other, after forking twice more, reached a depth of slighly more than 5 feet. Twenty-two primary laterals had originated at depths of less than 1 foot, and several small ones were found at deeper levels. Four laterals exceeded 15 feet in length, the longest being 19 feet. A fifth one measured 12 feet; the others were of various shorter lengths. The number of secondary branches varied from 40 to 75 on the 4 largest laterals. Horizontal secondaries as long as 6 feet were found, at which size tertiary branches of conspicuous size were present. None of the vertical secondaries, or sinkers, reached deeper than 5 feet.

The same general sequence of development is followed by the secondary branches as by primaries. Their early growth is largely manifested in elongation, most of the side branches being small, delicate, and ephemeral. When a length of 4 or 5 feet has been attained, permanent branches of the next higher order first become conspicuous. The same sequence may be repeated again by tertiary branches on still older root sytems.

Branches of the second or higher orders differ from the primary laterals in certain growth habits. The former frequently are found to oblique upward into the raw humus of the soil surface, whereas the latter usually run at slightly deeper levels. Also, branches of the higher orders often branch so extensively near the end that the parent root can no longer be identified. It is doubtful whether such a root can subsequently undergo any pronounced elongation. Laitakari (*17*) observed a similar situation in Scotch pine, and states that very little further elongation occurs. Primary laterals, on the other hand, always maintain their identity in a morphologically distinct tip capable of indefinite growth.

A specimen tree about 30 years old, 22.5 feet tall, and 3.5 inches d. b. h. may be cited to illustrate a more advanced stage of development. This tree grew on a rather high, sterile site, whereas the preceding one was located on lower, but nevertheless well-drained, ground. Though such minor variations in site do not seem to be reflected noticeably in root development, they cannot be wholly ignored in comparing the two trees. Excessive drainage on the higher site quite probably stimulated deeper penetration of the vertical roots.

The taproot of this tree reached a depth of 9 feet. Its upper part was strongly developed, being 5 inches in diameter 6 inches below the soil surface and 3 inches in diameter at a depth of 2 feet. At that level

it divided into 3 descending branches; these subsequently divided further. At the 5-foot level there were 15 of these descending branches distributed within a circular area about 2 feet in diameter (fig. 6). Most of them did not extend deeper than 7 feet, but the two longest ones reached depths of 8.5 and 9 feet. Growing tips were fairly abundant, and active mycorrhizae were collected as deep as 7 feet.

There were 20 sizable laterals, 13 of which originated at depths of less than 6 inches; the other 7 were found at greater depths down to the 2-foot level. The longest lateral measured 31 feet, another 29 feet, and at least 3 others exceeded 20 feet in length. The smaller ones included in the count were estimated to be about 8 feet long.

A few, of still smaller size, were not recorded. Secondary branches were not extensively excavated. One of the larger secondary laterals was 9 feet long, another was exposed for 11 feet without recovering the tip; several others were estimated to be of equal, or greater, length. One sinker was observed to end about 5.5 feet deep, but others probably reached deeper. The largest sinkers generally may be expected to approximate the taproot in depth.

FIGURE 6.—Taproot exposure of a 30-year-old tree. The excavation was 4.5 feet deep when the photograph was taken; it was subsequently extended to a depth of 9 feet to recover the deepest branches.

On any tree secondary branches that can be classified as large in proportion to all secondaries are relatively few. The majority of them are comparatively small. Some data taken from the last-mentioned specimen tree illustrate this point. The secondaries were tabulated in three size categories: (1) Length greater than 5 feet, (2) length 2 to 5 feet, and (3) length less than 2 feet. In most cases, the length was only estimated from the features of the basal part of the root, but as noted above, such estimations can be sufficiently accurate for the purpose. Of 348 secondary branches tabulated from 7 primaries, 22 were classed in group 1, 104 in group 2, and 222 in group 3. The most richly branched primary bore 95 secondaries, of which 6 were classed in group 1, 31 in group 2, and 58 in group 3. The smaller primaries usually had no secondaries falling in group 1.

GENERAL CONCLUSIONS FROM STUDY OF ROOT SYSTEMS IN EARLY STAGES

The plan of this paper thus far has been to present general morphological data from the developmental point of view. The growth of the root system has been followed from the seedling stages to the conditions found in immature trees 20 to 25 feet tall. Subsequent root development, as illustrated by larger trees, proceeds along somewhat different lines. Hence, certain generalizations applicable to the earlier growth stages are now presented.

Proportions of Vertical and Horizontal Secondary Branches

A summation of data with respect to the proportions of vertical and horizontal secondary branches is given in table 1. Since the horizontal category includes branches on both lateral sides of the parent root, it is evident that the primary puts out branches in three general directions, and that the sinkers represent approximately one-third of the total. This three-way distribution of branches cannot be correlated with the anatomy of the protostele. Although the primary xylem is not infrequently triarch in the roots of pitch pine, the diarch condition is most common.

Increasing Prominence of the Lateral System

Mention has been made above of the increasing prominence of the lateral system of roots as the tree develops. In the pine barrens, the taproot usually is the longest and most conspicuous root up to the eighth to tenth years of the plant's life. Soon after that time, the strongest laterals linearly outstrip the taproot, and eventually nearly all of them exceed the taproot in length. Mention also has been made of the tendency of the root system to flatten out and become relatively more localized in the topsoil as development progresses. This change comes about largely through the more rapid growth of the primary laterals as compared with that of the taproot and sinkers. A second, and less important, factor is the tendency of the secondary laterals to surpass the sinkers in growth rate during later development. This is evident only in trees 10 or more feet tall, and after the secondaries have attained a length of 4 or 5 feet.

TABLE 1.—*The proportions of vertical and horizontal secondary branches of three pitch pines*

Tree no.	Primary roots tabulated	Secondary roots				
		Total	Vertical		Horizontal	
	Number	*Number*	*Number*	*Percent*	*Number*	*Percent*
1 [1]	3	80	37	46	43	54
2 [2]	9	286	96	34	190	66
3 [3]	6	284	75	26	209	74

[1] An unusually vigorous 10-year-old sapling.
[2] 17-year-old tree cited in text.
[3] 30-year-old tree cited in text.

Table 2 illustrates these trends of development. The increasing size of the figures from top to bottom in the sixth and seventh columns

is most informative. The larger ratios shown by the last two figures in the ninth column are also undoubtedly significant; the other figures in that column probably only express variations around a norm of 1.0.

TABLE 2.—*Data illustrating the increasing prominence of the lateral system and the relative flattening of the entire system as the tree develops*

Height of tree	Age of tree	Depth of taproot	Depth of strongest sinkers	Length of longest primary lateral	Primary laterals longer than taproot	Ratio of longest primary lateral to taproot	Length of longest secondary laterals	Ratio of secondary laterals to sinkers
Inches	*Months*	*Inches*	*Feet*	*Inches*	*Number*		*Feet*	
1.5	2-3	5		1.5		0.3		
	Years							
3.0	1	8		3.0		.4		
11	4	18		15		.8		
22	8	27		33	(1)	1.2		
Feet		*Feet*		*Feet*				
2.7	10	3.3	1	5.5	3	1.7	1	1.0
4	12	4	3.5	8	5	2.0	2.5	.7
4.5	11	2.5	3	12	13	4.8	4	1.3
7	10	3	3	14	12	4.7	2.5	.8
7.5	14	3	4	10.5	12	3.5	3.5	.9
14	17	5	5	19	12	3.8	6	1.2
22.5	30	9	7	31	17	3.4	12	1.7

[1] No data.

RELATION OF LATERAL SPREAD OF ROOTS TO HEIGHT OF TREE

A simple criterion for estimating the area occupied by the root system would be of some practical value. However, the limitations of such a criterion must be recognized. Because of inherent variations, and variations due to the environmental complex, close estimations of root development are impossible when based only on aboveground characters. In the pine barrens, about four of the laterals may be expected to exceed the trunk of the tree in length. The longest lateral usually is approximately 1.5 times as long as the stem. This applies only to trees less than 25 feet tall and to trees that have not been forced to spindle upward. Data illustrating this generalization are given in table 3.

It is even more difficult to estimate the depth of taproots. The figures in the first and third columns of table 2 indicate that the stem and taproot attain linear equality somewhere between 2 and 4 feet. Previously, the taproot was longer; after the interval of equivalence, the stem is always longer. Nine feet appears to be near the maximum depth attained by pitch pine taproots in the locality.

TABLE 3.—*Data showing the relation of the maximum lateral spread of roots to height of tree*

Height of tree	Laterals exceeding the stem in length	Length of longest lateral	Ratio of longest lateral to height of tree	Height of tree	Laterals exceeding the stem in length	Length of longest lateral	Ratio of longest lateral to height of tree
Inches	*Number*	*Inches*		*Feet*	*Number*	*Feet*	
11		13	1.2	2.8	4	6.3	2.3
11		15	1.4	4.0	4	8.0	2.0
18		26	1.4	4.5	7	12.0	2.7
18		27	1.5	7.5	2	9.0	1.2
20		40	2.0	7.0	3	14.0	2.0
				7.5	4	10.5	1.4
Feet		*Feet*		14.0	4	19.0	1.4
2.5	4	4.7	1.9	22.5	6	31.0	1.4
2.8	5	5.3	1.9				

Relation Between Root Diameter and Length

Little has been said in the preceding discussion concerning the diameters of roots. From a physiological standpoint, diameters are of far less significance than lengths of roots and volume of soil occupied. Basal diameters, particularly those of horizontal roots, are roughly proportional to lengths, and can be used to some advantage in estimating the lengths of laterals. In cases where there is appreciable enlargement at the point of origin, measurement should be taken beyond the region of pronounced tapering. For a general estimate of the length of a root, the basal diameter is measured in millimeters; the length expectancy is an equivalent number of feet. For example, a root of 5 mm in diameter at the base is about 5 feet long; a root 30 mm in diameter at the base is about 30 feet long. Naturally, there are rather wide variations, with a somewhat greater tendency of lengths to fall short of, rather than to exceed, the expectancy according to this simple formula. The length is generally inversely proportional to the degree of branching, i. e., the parent root is shorter when well branched than when poorly branched. The diameters in millimeters and the lengths in feet were tabulated for 53 primary laterals from seven trees. The ratio of the length figure to the diameter figure was calculated for each root. The average of these ratios was 0.85. This expresses the tendency, noted above, of lengths to fall slightly short of the simple ratio of 1.0.

Figure 7.—Exposure of part of the root system of a 17-year-old pitch pine showing the ropelike character of the laterals. The tools mark the ends of roots. The root on the right was 19 feet long.

It can be seen that these lateral roots are rather slender structures. Since branches usually are distinctly smaller than the parent root, and on older roots are relatively sparsely distributed, the larger laterals may aptly be described as "ropelike" in appearance (fig. 7).

During the younger stages sinker roots maintain the same proportions as laterals, but after they reach a depth of 3 or 4 feet, elongation

is retarded. Growth in diameter may continue, resulting in greater thickness in relation to length. The diameter of a taproot furnishes no reliable indication of its length.

ROOT DEVELOPMENT OF TREES APPROACHING MATURITY

As has been mentioned, root development proceeds along somewhat different lines as the tree approaches maturity. The size at which this change occurs may be designated, more or less arbitrarily, as follows: Height, 25 feet; diameter breast high, 4 inches; radial spread of roots, 30 to 35 feet; and age, 30 years. Early root development involves a rather steady increase in length, both laterally and vertically, during which the ropelike character of the laterals is maintained without marked thickening at any point and general correlations exist between extent of root systems and size of tops and between diameters and lengths of roots. Subsequent growth adds little or nothing to the radial spread of the root system. Büsgen and Münch (*4*), in a footnote, indicate that a similar sequence of development has been observed in Europe (probably on *Pinus sylvestris*). Instead of further elongation, a marked thickening becomes apparent along the basal 1 to 3 feet of the stronger primary laterals. The smaller primaries, and to some extent, the secondary laterals, continue to elongate, thus increasing the density of roots within the 30-foot radius. Ultimately, the originally smaller primaries attain lengths approximately as great as those of the strongest laterals; they then cease elongating and undergo basal thickening. Further penetration of the subsoil by the taproot and the strong sinkers near the root crown practically ceases, downward growth being confined almost entirely to branches of those roots and to the younger, more distal sinkers.

FIGURE 8.—The central roots of a mature pitch pine, showing the strong development of sinkers associated with a weak taproot. The longer roots reached a depth of about 8 feet. The tree was 48 feet tall, 9 inches d. b. h., and 85 years old.

The taproot of pitch pine, which is nearly always a conspicuous feature of younger root systems, becomes relatively insignificant on some older trees. In such cases, the function of anchorage is taken over by several of the innermost sinkers, which have become much thickened (fig. 8). The size of these sinkers varies inversely with the size of the taproot.

The basal thickening of the primaries very frequently is more pronounced in the vertical plane, resulting in "planklike" roots. The asymmetry rarely extends outward more than 3 feet. Undoubtedly, it is a mechanically efficient means of bracing the tree against the increased stresses of a larger crown. Rigg and Harrar (*22, p. 396*) noted an extreme development of this tendency in the shallow root systems of trees growing in sphagnum peat, and, apropos of the mechanical efficiency of such modifications, state that "the strength of beams of varying transverse sectional shapes approximates the square of the vertical dimension when the horizontal dimension remains the same."

These planklike roots frequently are decidedly eccentric; that is, the morphological center of the root is below the actual center, as a result of greater thickening on the top than on the under side (fig. 9). Thus, shallowly situated laterals often grow out of the ground near the stem base, producing the familiar buttressed effect. Although many deciduous trees display this feature more markedly, it shows plainly in pitch pine and probably is a wide-spread habit. Laitakari (*17*) mentions it in discussing the roots of *Pinus sylvestris*. Vertically narrowed roots and buttresses attain striking dimensions in some tropical trees. Obviously, buttresses are mechanically efficient structures; the same amount of material would give greater support in that position than in the strictly horizontal position of the original root. Investigations of tropical trees indicate, however, that buttresses cannot always be explained as a simple response to mechanical stress. They may develop when lateral stresses are negligible. Air and water relations appear to be influential factors (*7, 9*). The formation of the characteristic buttresses of the bald cypress (*Taxodium distichum* (L.) Rich.) has been shown to be dependent on a combination of water plus air (*16*).

FIGURE 9.—Cross-sectional views of four lateral roots cut close to the the taproot. All show narrowing in the vertical plane, and the two lower ones show eccentric thickening on the upper side.

The buttresslike development of lateral roots of mature, erect trees suggests that the roots function mechanically as props or braces,

and that the thickening develops in direct response to the increasing stresses produced by the growing crown. Undoubtedly, supporting roots also function mechanically as tensile guy wires, but that role is minor and is secondary to the propping action. Laitakari (*17*) has pointed out the tendency of Scotch pine to produce more and stronger laterals on the side opposite that of the prevailing winds, i. e., in the propping position. To check this tendency further in pitch pine, leaning trees were examined. The one-sided stress on the roots of such individuals is distinct and constant. Of 4 such trees examined, 3 showed a very marked concentration of laterals on the side under the inclined trunk, with no sizable surface laterals on the opposite side (fig. 10). This indicates clearly that those roots in the propping position are thickened in response to stress; those in the guy-wire

FIGURE 10.—Exposed roots of a leaning tree, showing the majority of the lateral roots in the propping position.

position do not thicken, but may possibly break under the strain, die, and disappear.

Examination of the roots of the fourth leaning specimen, in which there was no correlation between lean and root development, did not weaken this conclusion. It was a sprout, only slightly inclined and relatively small (2 inches d. b. h. and 15 feet tall). The symmetrically arranged root system had been laid down by the parent tree; the sprout had not yet become old enough or heavy enough to alter its symmetry appreciably.

The positive correlation between the growth of roots and of tops has been pointed out by numerous investigators. It is practically regarded as axiomatic by most students of plant roots. The subject was not given particular attention in this investigation. However, the leaning trees discussed above were all rather weak, slow-growing

specimens. The number and size of the primary laterals and the thickness of the taproot were distinctly inferior in comparison with those of normal trees of the same size. In view of the open character of the forest, it is doubtful that the weakness of the specimens was due to suppression by larger trees. It seems most probable that both the leaning and the general weakness of the tops had resulted from, or were coincident with, an originally weak root system.

It seems quite probable that the processes of maturing and senescence are correlated with the tree's inability to extend the root system into new territory. It is obvious that the demands for support placed upon the inner root system increase enormously as the top attains mature stature. The material requirements for strengthening near the root crown may be an important factor in precluding further root extensions. In any event, a fertile field for speculation is offered by the question of whether maturing and senescence are wholly internal and protoplasmic, with the cessation of root extension as a result, or whether the mechanical difficulties of support and of transportation through an ever-widening root system are contributory causes of those processes.

ORIGIN OF ROOT BRANCHES

The endogenous origin of the branches of roots is one of the fundamental facts of plant anatomy. Normally, branches emerge along the maturation zone of the root tip, and along the tender parts farther back which have not undergone marked secondary thickening. The completion of a solid ring of secondary wood generally precludes further branching. On an actively growing root, this probably takes place during the second year; on roots growing less vigorously, either of two types of behavior may occur: (1) Secondary thickening may be indefinitely delayed, and the period of possible branching equally prolonged; (2) secondary thickening may advance to within 5 mm of the tip, resulting in thick, blunt-ended roots with a barely perceptible tip of tender tissue. The former is usually associated with dormant lateral roots; the latter condition is most often found in vertical roots deep in the subsoil.

The growing tips of the major extending roots are thick and succulent, about 3 mm in diameter. The tips of branches at emergence usually are distinctly smaller. In cases where the parent tip has died (a surprisingly common occurrence), a replacement tip emerges which, from the start, has the attributes of the parent root. It resembles the latter in size and vigor of growth, and at once assumes a forward rather than a lateral direction (fig. 11). Sometimes two such tips emerge opposite each other; the immediate result, while the dead parent tip persists, is an apparent trifurcation; ultimately the dead root disintegrates, leaving a bifurcate condition.

The formation of replacement tips was not observed to take place when dying-back had extended into the region of complete secondary thickening. In that event, an existent branch may undergo greater than average growth and after the disintegration of the dead parts appear to be the normal continuation of the parent root. This is nearly always what has happened in those occasional cases in which a major lateral root appears to become vertical, or vice versa. The abrupt change of direction marks the origin of a branch which assumed dominance after the death of the parent root beyond that point.

The roots of many herbaceous plants, which do not undergo extensive thickening, may produce branches at any point. Moisture frequently stimulates abundant branch production on such roots in regions far removed from the growing tip. The fact that the woody root, in some species at least, is only slightly or not at all capable of such response warrants considerable emphasis, particularly in respect to the taproot. With such species, of which pitch pine appears to be one, it means that the original complement of primary branches on the seedling taproot never can be increased in later life. It is highly probable that an originally poorly branched taproot, or one deprived of its branches in any way, will result in a weak root system and predispose toward general weakness of the tree. The best cultural care is, to some extent, wasted on a tree with an inadequate root system. Of course, when the number is reduced, roots may become somewhat larger and branch more profusely than usual, but it is doubtful that such development can be fully compensatory in respect to absorbing area. Certainly, reduction in the number of primary laterals weakens the support of the tree and predisposes toward wind throw in later life.

FIGURE 11.—A young replacement tip developing after the death of the terminal portion of the parent root. Note its relative size and direction of growth in comparison with the remnants of normal side branches.

The above statements do not apply with equal force to all tree species. Adventitious roots, arising both from stems and from other roots, are mentioned occasionally in the literature. Luncz (*19*), in reviewing some European work, cites Austrian pine and cherry as examples of trees whose roots may give rise to adventitious branches, and states that such branches are not morphologically distinguishable from the original roots. If adventitious roots ever arise on either pitch or shortleaf pines, they must require a more powerful stimulus than normally occurs in nature. Examination of scores of roots showed that branches of all sizes, on both species, universally extended inward to the protoxylem of the parent root, and therefore had originated when that region was young (fig. 12). The only possible exception found was in cases where the roots had been severed smoothly with a sharp instrument. Some small branches had subsequently arisen exogenously near the cut ends. Examples of this were found at the scene of stump-grubbing operations of the preceding winter. No observations were made of exogenous branches of greater age.

GROWTH OF ROOTS UNDER WATER

Probably the most striking single feature of pitch pine roots observed in the course of this investigation is the fact that they develop extensively below the water table in saturated soils. So far as the writer is aware, no similar observation on a mesophytic tree has been reported. Such hydric species as *Taxodium distichum* undoubtedly root under water, but in practically all descriptions of the roots of mesic trees, and even of such bog inhabitants as American larch and black spruce, they are reported to develop entirely above the saturated zone. Textbooks generally state that root penetration ceases when the water table is encountered (*4*, *24*), and much has been written of the deleterious effects of poor drainage on various economic plants. Rigg and Harrar (*22*) found that none of the six conifers growing in the bogs of Washington extended roots below water. According to Woodroof (*30*), the roots of the pecan cannot tolerate submergence. The longleaf pine (*Pinus palustris* Mill.), which in some respects is the southern ecological equivalent of pitch pine, is reported by Heyward (*12*) not to penetrate below the water table on poorly drained sites. However, Hesselman (*11*) notes the occurrence of vigorously growing spruce stands in Sweden in the vicinity of springs, even where the water level comes to the surface. Although no data are given on root development, the trees obviously do produce roots under water in such situations. Adamson (*1*) describes an Indian tree (*Terminalia arjuna* Bedd.) which inhabits

FIGURE 12.—Cross-sectional views of roots showing the central origin of branches: *A*, A section from a taproot, 5.5 inches in diameter; *B*, section from a very small primary lateral, shown about natural size.

river banks and extends roots out into the saturated soil of the river bed.

The root behavior of white cedar (*Chamaecyparis thyoides* (L.) Britton, Sterns, and Poggenberg), which is the typical bog tree of southern New Jersey, has not been investigated, but in view of the sites supporting it, it probably does root below the water level. Pitch pine, however, is most at home on higher, well-drained sites, and intermingles only slightly with the white cedar in the narrow dividing ecotone. The pine can live among the cedars only by overtopping them; once the cedar canopy closes, any pine beneath it is doomed. The occasional pine that towers among the cedars points clearly to the fact that it is not the site itself, but the competition, that generally excludes them.

Pitch pine is to be regarded as a member of that wide-spread plant fraternity which, demanding little except a place in the sun, must find its place as a rule in the left-over, unfavorable areas that will not support the "nobler" species. Such are the sterile, fire-scorched sands of the Coastal Plain, or the wind-swept ridges of the mountains, both of which are typical pitch pine sites. It invades burned-over areas, abandoned fields, and clearings, where individuals may persist, but where, if conditions are favorable, reproduction soon becomes impossible in competition with other species. Although its tolerance of competition is low, its tolerance of a wide range of site factors is remarkable. Sandy soils or clay, fertile or infertile, drained or saturated, situations xeric or mesic or hydric—all are acceptable to this undiscriminating species.

Three trees were examined with respect to root growth below the water table.[6] The largest one was 33 feet tall and 5 inches d. b. h. It was located on a steep incline that bounded the valley of a creek. The water table was bout 3 feet below the surface at the spot where the tree stood. Lateral roots extended up the incline into typical Lakewood sand; on the lower side, they extended into the flat valley bottom where water was only about 6 inches below the surface, with sphagnum moss and cinnamon fern (*Osmunda cinnamomea* L.) to testify to the saturated condition. The laterals were of the usual type, and put down sinkers regardless of the proximity to water (fig. 13, *A*). They were aligned generally parallel with the incline of the soil surface, both uphill and downhill. The taproot penetrated the zone of saturation as a strong shaft, 4 inches in diameter at the level of submergence. It broke up into a fan-shaped mass of branches along its fifth foot. This fan, which was about 2 feet wide and 4 to 6 inches thick, was a most peculiar tangle of roots of various sizes, unlike anything found on drained soils (fig. 13, *B*). As a mass, it ended at a depth of 6.5 feet, though a few individual roots reached slightly deeper. Many of the tips were alive and growing slowly.

A large proportion of the ultimate fine branches were mycorrhizal,[7] a condition which was quite unexpected. The literature contains

[6] The method of securing the central root systems from saturated soils was as follows: The topsoil was removed around the root crown down to the water, and the lateral roots were severed. A strong pole was chained to the stump and operated as a lever over a fulcrum of logs. With this, 3 or 4 men could pull stumps of the sizes described. Practically every root was secured intact, as the sand was held enmeshed in the root tangle and was lifted as a solid mass.

[7] The true mycorrhizal character of these roots has been verified by K. D. Doak, of the Allegheny Forest Experiment Station.

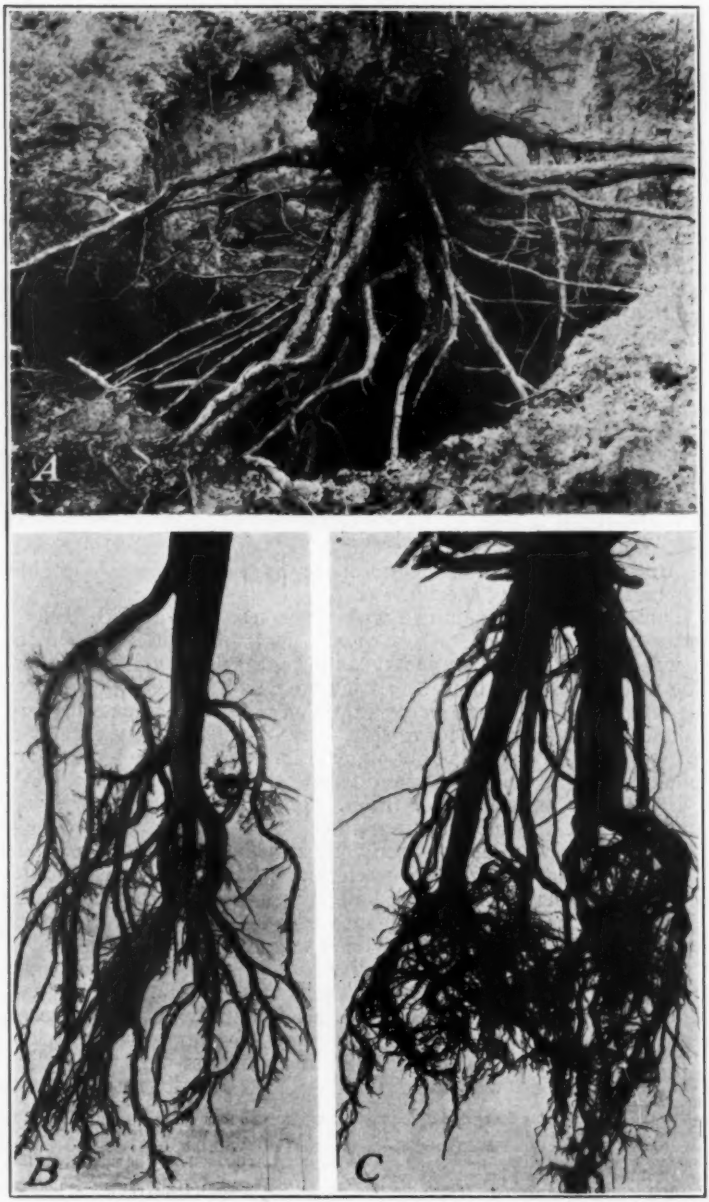

FIGURE 13.—Central roots of trees from saturated soil: *A*, Preliminary excavation of the largest tree, with water standing in the bottom of the hole. *B*, Under-water portion of the taproot of the same tree. It reached a depth of 6.5 feet. *C*, Roots of a smaller tree, from a site where the water table was only 8 inches below the soil surface; these roots reached a depth of 5 feet.

very few definite statements with respect to the occurrence of mycorrhizae in nature on submerged roots. The negative findings of Bondois (*2*) constitute the only direct observations on this topic known to the writer. The fact that mycorrhizae had not been reported on submerged roots and that good drainage seems essential for the successful synthesis of mycorrhizae in culture (*21*) has led to the general opinion that they do not develop under saturated conditions. In some respects the presence of mycorrhizae far below a permanent water table is even more remarkable than the growth of the pine roots themselves. Interesting questions are raised as to the identity and physiology of the fungus involved—questions to which there are at present no answers.

The other two trees taken from saturated soils grew on a lower site, in what probably was originally the ecotone between white cedar and pine stands. (Clearing had disturbed the natural vegetation.) The water table was about 8 inches below the surface in dry weather; for several weeks after a rainy period it remained only about 4 inches down. The site was not a true bog as known in glaciated regions. The surface soil to a depth of 6 to 10 inches was composed largely of fibrous plant debris, hummocks of cinnamon fern, and sphagnum moss. It was densely interwoven with living roots and rhizomes. This fibrous mat rested on sand, with a sharp line of demarcation.

The specimen trees were 4 inches d. b. h. and about 20 feet tall, somewhat smaller than the one discussed above. Both were vigorous and healthy in appearance, the more recent internodes being 12 to 18 inches long. One of these trees was essentially taprooted, though the shaft forked into two almost equal and parallel parts and divided extensively into a fan-shaped mass of many-branched and contorted roots (fig. 13, *C*). It ended at a depth of 4 feet, with a few individual roots going about 1 foot deeper. The finding of two such fans indicates that their formation is a normal response to the saturated condition. Why this is the case must, at present, be left to conjecture. The branches did not originate wholly on opposite sides of the taproot, which would seem to eliminate diarchy of the protostele as an explanation. In fact, many of the roots were triarch. This tree, also, showed numerous growing tips and mycorrhizal clusters.

The second smaller tree had no taproot, but was anchored by four strong sinkers located a few inches out from the stump. They ended at depths of 2.5 to 3 feet. One of these obliqued downward at an angle of about 45°, which is notable as the only example found on any tree of a major root following such a course. (Major roots are almost always either definitely vertical or definitely horizontal.) This tree had developed no fanlike structure.

These three trees demonstrate that pitch pine can thrive on saturated soils; that it will root solidly and deeply on such soils, with no more than the usual danger of windthrow; and that mycorrhizae can develop under conditions of saturation.

Before applying these conclusions generally, it will be necessary to examine trees in other regions and on other soil types. It is possible that conditions in the pine barrens permit a greater degree of aeration of ground water than usually occurs elsewhere. As is well known, many herbaceous plants that are intolerant of saturation will grow in water culture if properly aerated. Hole (*14*) has shown that some trees, at least, respond in the same manner. Hesselman (*11*) attributes the growth of spruces in the saturated soils near springs to the

unusual aeration of the water. Further investigation is necessary to determine whether it is the species or the site that is most unique in southern New Jersey.

Plant roots growing under water often show anatomical differences, particularly in the development of air spaces. Adamson (*1*) reports the presence of lacunae in both the primary and secondary cortex of the submerged roots of *Terminalia arjuna*. However, no air spaces, nor marked looseness of the cortical cells, can be discerned in the submerged roots of pitch pine. Hesselman (*11*) mentions that neither pine nor spruce roots develop special air passages when submerged. Bondois, in a paper dealing with anatomical features of the roots of mesic trees grown in water (*2*), noted that spruce (the only conifer studied) did not develop cortical air spaces, though this did occur in several hardwood species.

ROOT DEVELOPMENT ON HEAVIER SOILS

Conditions did not permit extensive comparative studies of *Pinus rigida* on widely different soil types, though such studies are much to be desired. Some observations and partial excavations of root systems were made near Mont Alto, Pa., where pitch pine grows on the higher ridges in the mountains. The soils are raw, podsolized, stony, and high in clay. They are rendered so sticky and compact by the clay that spading is almost impossible without preliminary loosening with a pick. These soils offer far greater physical resistance to root penetration than do the sands of the Coastal Plain and in all probability retard root elongation irrespective of other factors.

Two small trees, 11 and 14 feet tall, were examined. The following differences, in comparison with New Jersey specimens, were noted:

(1) Vertical roots were generally weaker in both thickness and length. Taproots of these trees reached depths of 3 to 3.5 feet.

(2) Primary laterals were shorter, the longest ones scarcely exceeding the stem in length.

(3) Laterals frequently ran closer to the soil surface than in the New Jersey sands. This probably is a result of the greater moisture-retaining capacity of the clayey soil.

(4) Laterals followed more tortuous courses through the soil owing to the resistance of the soil itself and to obstructing rocks.

(5) Secondary branches were generally more numerous per linear unit of primary root.

(6) Primary laterals had undergone much more basal thickening than is usual on New Jersey trees of the same size. This is undoubtedly correlated with the weakness of the taproots and the consequently greater burden of support placed upon the laterals. Stronger prevailing winds, also, may be a factor.

These generalizations, though derived from a limited number of observations, are in essential agreement with statements in the literature relating to tree roots. Hence they carry more weight than could be granted to them if they were not thus supported. Of particular interest are the observations of Rigg and Harrar (*22*) on the roots of conifers growing in sphagnum peat. They found markedly greater root elongation in this loose substratum than in more compact and resistant soils. They agree with Büsgen and Münch (*4*) that mechanical obstructions rather than conditions of nutrition retard root elongation.

ROOT FUSIONS

Frequent references are made in the literature to root fusions, both within the same system and between different systems. The occasional persistence of life in coniferous stumps is usually explained as resulting from intersystemic fusions with the roots of a living tree (fig. 14). In this investigation, such natural roots grafts were found only rarely, and with one exception involved root of the same tree. Some were found on both pitch and shortleaf pines. Close aggregation of trees is most conducive to root grafts. The fact that such situations were avoided in choosing specimen trees probably accounts for the small number observed.

FIGURE 14.—A natural root fusion on *Pinus echinata*.

ABSORBING AREA AND THE PROBLEM OF ABSORPTION

This report is concerned primarily with the gross features of root systems. The description and interpretation of the ultimate branching patterns and of functional areas of absorption entail more detailed study, involving tip-by-tip analyses and laboratory experimentation.

In a general way, the terminal parts of vertical roots are coarse and sparsely branched (fig. 15); those of horizontal roots are more slender and much more profusely branched. On vertical roots, the diarchy or triarchy of the protostele often can be determined readily by the alinement of the branches (fig. 16); on horizontal roots, the alinements usually are obscured by the profusion and contortion of the branches. Roots feeding in the surface humus are most intricately branched; the network often is so complex that it is next to impossible to obtain any sizable portion intact and free from organic debris. The intricacy is enhanced further by innumerable mycorrhizal clusters. Though mycorrhizae are found at all levels (fig. 15), they occur in greatest abundance in the surface organic layers of soil.

The amount of functional absorbing area is, in the final analysis, one of the most significant features of root systems. The extent and degree of branching of the skeletal framework of a root system is important only insofar as it brings the absorbing membranes of young tips into contact with the necessary water and nutrients. Exact knowledge of the root parts wherein absorption occurs is very meager. It is not known with certainty how much of the terminal region of a root actively absorbs, or whether dormant tips can absorb, or whether mycorrhizae are important as absorbing organs. According to the usual concept, absorption takes place primarily in the zone of extension found between the apical meristem and the suberized mature region of a growing root. Scott (*23*) has reiterated this general idea,

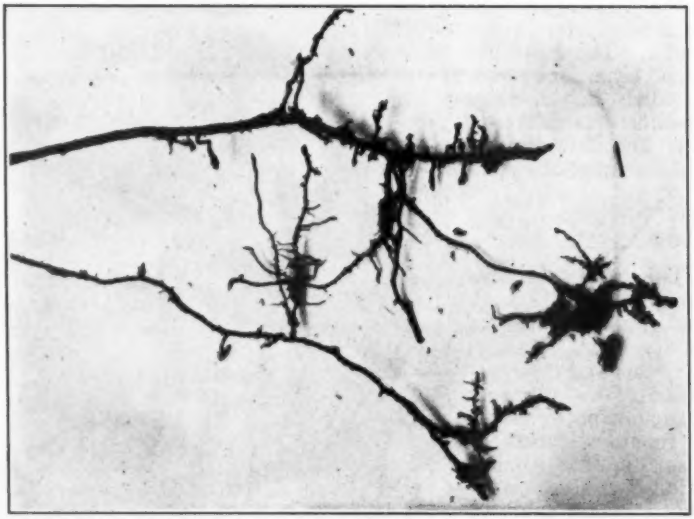

FIGURE 15.—Tip regions of two vertical roots taken 7 feet deep in drained soil. The clusters are mycorrhizae. Note the paucity of permanent branches.

and pointed out that, with retarded growth due to drought or other unfavorable conditions, suberization processes continue and encroach on the absorbing regions of the root. The tip ultimately may be completely enclosed by a suberized covering.

It has been observed by various investigators, and verified by the writer in respect to the species studied, that the roots of pines do most of their growing in the spring and fall. Finding a growing tip was a rare occurrence in July, August, or early September. The dormant tips found generally during that period were brown and shrunken as compared with growing ones. Free-hand sections showed the cortical cells to be in a flaccid condition, and the cell walls were brown inward to the endodermis. They appeared to represent thoroughly that state described by Scott wherein the root tip is completely enclosed by a suberized covering. If it is admitted that active absorption takes place only in growing tips, and if Scott's statement is accepted that

"the distribution of suberization may be used as an indication of the delimitation or curtailment of the absorbing region of the root", then the incongruous situation is presented of absorbing area being reduced to a minimum when transpiration stresses are greatest. Scott supplies no answer to this question. Perhaps that actually is the case, and the tree obtains its water during the late summer months solely through the few vertical roots whose tips are not completely dormant. Though many of the tips found deep in the subsoil also are dormant, and the number of growing ones seems wholly inadequate to supply the needs of the tree, the fact remains that the tree passes through the dry summer period annually without visible deleterious effects. The prevailing paucity of root hairs on pitch pine roots renders the mode of water entry even more uncertain. The whole problem of the location and mechanics of water absorption in the pine offers a fertile field for further research.

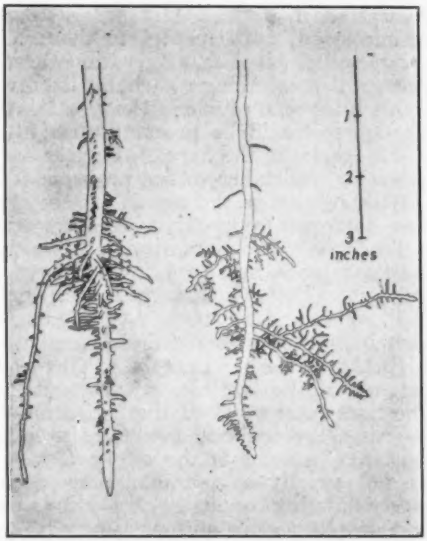

FIGURE 16.—Line drawing of two tips of vertical roots. The one on the left was triarch and that on the right, diarch.

SIGNIFICANCE OF THE ROOT HABITS OF PITCH PINE

The relation between initial root habit and the ability of seedlings to maintain themselves on various sites has been emphasized by Toumey (*25*) and is generally recognized by foresters and ecologists. To establish itself on a xeric site, the seedling must promptly make contact with the more permanent reservoir of moisture in the subsoil. Those species which push their outposts farthest into the midwestern prairie are notably deep-rooted. *Quercus macrocarpa* Michx. and *Juglans nigra* L. may extend their taproots down more than 4 feet during the first season of growth (*13*). Species with inflexibly shallow roots are restricted to sites where the topsoil is always moist.

The excessively drained sands of the pine barrens constitute a relatively xeric situation; hence the native pitch pine might be expected to put down a vigorous taproot during the first season. As noted in the discussion of seedling root systems, 1-year-old plants reach a depth of 1 foot or more on exposed situations. Although this is not a striking development, it does bring the roots into contact with sands wherein the water content only very rarely is depleted to the point of the wilting coefficient. In comparing this growth with that of xeric broad-leaved trees of the Middle West, it must be borne in mind that the latter undoubtedly transpire much more water than

do needle-leaved seedlings in the more humid atmosphere of the eastern seaboard and therefore require a deeper and more extensive root system.

The literature indicates that seedlings of pines, in general, develop deeper root systems than most other native gymnosperms, but, at best, fall far short of the growth attained by such hardwoods as black walnut and the more xeric oaks and hickories. *Pinus palustris*, growing in the southern sandy Coastal Plain, ordinarily does not penetrate the soil deeper than 1 foot during the first season (*18*). *P. ponderosa* Laws., the dry-land tree of the Rocky Mountain area, does not develop unusually deep seedling root systems (*8*). Evidently the successful ecesis of pines on dry areas cannot be wholly ascribed to root adaptations. Other features, including reduced transpiration, undoubtedly are significant. Pitch pine is equaled or surpassed, with respect to depth of seedling root systems, by several other pines and by various hardwoods. From an ecological viewpoint its seedling root habit is only one of the complex of characters by means of which the plant is adapted to xeric situations. Though it would be presumptuous to account for the prominence of the species in the flora of the pine barrens on the basis of any one character, resistance to fire probably is most decisive (*20*).

With respect to the extent of root systems of mature trees, pitch pine conforms generally with other species for which information is available. The similarity between the root systems of pitch and shortleaf pines has been noted above. The root development of jack pine (*Pinus banksiana* Lamb.) evidently closely parallels that of *P. rigida* and *P. echinata* (*6*). Longleaf pine and Scotch pine, at least under certain conditions, may considerably exceed pitch pine in spread of roots. Lengths of 70 to 80 feet are reported (*12*, *17*). Laterals of the bur oak attain lengths of more than 60 feet (*29*). The horizontal roots of the pecan may extend laterally 30 feet on 12-year-old trees, and generally maintain lengths of about twice the lateral spread of the crown (*30*, *31*). Such figures, of course, are not strictly comparable, since the various species grew under widely differing conditions of soil and climate. They show, however, that despite soil conditions generally conducive to root elongation the extent of root systems of pitch pine is not exceptional. Furthermore, they show the fallacy of the old notion that the spread of root systems of trees approximates the spread of the crowns. In trees beyond the sapling stages, the spread of roots is nearly always at least twice, and in many cases four or more times that of the tops. Equivalence of spread above and below ground is to be regarded as the exception rather than the rule.

Depth of roots is more generally responsive to soil conditions than is their lateral spread. The figures reported for pitch pine in this paper certainly do not approach the maximum depths attainable by tree roots. Even on the conservative basis of a depth of 8 feet and a lateral spread of 30 feet, the root system of a tree is seen to occupy an enormous volume of soil. Trees in nature nearly always are spaced so closely that considerable intermingling of root systems results. With these are associated the roots of all the subdominant shrubby and herbaceous plants of the forest floor. In general, the underground parts of the plants of the forest are more intimately

associated with each other than are the tops. Particularly on dry or infertile soils, underground relations probably determine the density of the vegetation. When viewed with a full appreciation of the extent and interrelations of root systems, it may justifiably be contended that the complexity of the forest community attains its greatest expression underground.

From a silvicultural standpoint, several suggestions are warranted on the basis of this study. They may be listed as follows:

(1) Only seedlings with strong, well-branched root systems should be planted. A root system poorly branched in early life will always be weak, both as a supporting and as an absorbing structure, and will result in a weak tree.

(2) Pitch pine is not recommended for planting in shallow soils overlying solid rock. The tendency to develop fairly deep vertical roots is strongly inherent in the species. Complete inhibition of the vertical development would result in weakly anchored trees, and probably would cause physiological disturbances.

(3) In the sandy soils of New Jersey, pitch pine may be planted on sites where the water table is as close as 8 inches to the soil surface. Development of vertical roots under such conditions is not markedly inhibited, and the trees are firmly anchored. Planting on sites where the water table remains at the soil surface is not recommended without further study. Aeration of the surface soil where the majority of the lateral roots are found may be essential to the health of the tree. Also, planting on heavy soils with a high water table is not recommended until the reaction of the species on such areas has been investigated.

(4) Pitch pine probably can best be used in mixture with other species. The natural oak-pine mixtures characteristic of the pine barrens constitute a pertinent suggestion. All observations indicate that the greatest productivity of the soil is to be realized by following this hint from nature. Pitch pine roots extensively, but not intensively. Some intermingling of the roots of adjoining trees may take place without initiating marked competition between them. In the case of such intolerant species as pitch pine, crown closure does not necessarily indicate complete closure underground. The mixed planting, in which the intolerant pine is given a start over more tolerant associated species, thus promises the greatest return.

COMPARATIVE OBSERVATIONS ON PINUS ECHINATA

Shortleaf pine (*Pinus echinata*) is an important component of the forest on drained soils of the Lebanon area. It occurs in mixture with *P. rigida* and the oaks, or, locally, in almost pure stands. However, it does not follow the pitch pine into low areas of poor drainage. Presumably, it cannot tolerate a high water table. In the Lebanon area, shortleaf pine appears to be even more intolerant of reduced light than pitch pine. The trees are remarkably well self-pruned, and if at all crowded tend to become spindling and weak.

Although this investigation was centered around pitch pine, observations sufficient to establish certain similarities and differences between the two species were made. It has been pointed out that no constant differences are apparent in the younger stages of growth

(up to 10 years of age). Differences are apparent in the roots of trees approaching maturity, but the stage of development at which those differences become conspicuous was not determined. The most striking difference is in the development of the taproot. *Pinus echinata* produces a much more powerful and massive central shaft (fig. 17) that maintains its thickness to greater depths and displays less tendency to divide into an array of descending branches. The depths attained by the vertical roots of the two species are essentially the same.

The root systems are similar in general form and extent. The same general relationships between spread of roots and height of tops, and between lengths and diameters of laterals, seem to prevail as in pitch pine. Also, the growth of laterals follows the same sequence, i. e., elongation and the maintenance of a ropelike character during the first decades of the tree's life, followed by retarded elongation and the initiation of basal thickening as the stresses incident to an enlarging crown increase. Thickening, however, is less pronounced in shortleaf pine, inasmuch as the taproot has assumed the greater burden of support, and for the same reason strong supporting sinkers near the root crown are few or wanting.

FIGURE 17.—Taproot of an 85-year-old shortleaf pine, showing its massive character.

These comparative generalizations concerning *Pinus echinata* are based on examinations of three specimen trees. Two of them were somewhat weak and spindling, 25 feet and 31 feet tall, respectively, and 4 inches d. b. h.; the third was a mature tree, 8.5 inches in diameter, 45 feet tall, and about 85 years old. It may be noted, incidentally, that the longest root excavated during the entire investigation was found on the somewhat atypical 25-foot specimen of *P. echinata*. The taproot of this tree gave off only 2 sizable laterals, the larger of which was 10 by 5.5 cm in cross section at the base, and 50 feet long. The root was unusually well branched; obviously, this was somewhat compensatory for the paucity of primary laterals. It is very doubtful, however, that the 2 primary laterals, even though exceptionally well developed, could equal the usual complement of 15 to 30 primaries in absorbing area. Certainly such a root system is mechanically weak as a structure for anchorage and support.

The suggestions given for silvicultural management of pitch pine hold equally well for shortleaf pine, except that this species cannot tolerate situations where the roots reach saturated soil.

SUMMARY

The root system of *Pinus rigida* was studied by the direct, dry method in the pine-barren section of New Jersey. It is to be classed in the generalized type. It attains a moderately extensive development both vertically and horizontally. The basic plan consists of a taproot from which 15 to 30 horizontal branches originate and extend radially in the surface layers of soil. From these primary branches horizontal and vertical secondary branches develop; these in turn give off tertiary branches, and so on. Normally, the root system occupies a roughly circular area of topsoil; in all of this area except the marginal parts it has extensive contacts with the subsoil through the development of vertical, or sinker, roots.

Seedlings are prominently taprooted. They reach depths varying from 3 to 12 inches during the first season of growth.

The taproots of seedlings 4 to 5 years old reach depths of 15 to 24 inches, and the strongest laterals approximate the same length. Plants of this age are semidecumbent; a permanent crook persists at the ground line long after they have become rigidly erect.

The taproots of plants 8 to 9 years old reach depths of 1.5 to 2.5 feet, and the strongest laterals approximate the same length. Plants of this age usually are erect.

Saplings 12 years of age are beginning to display regular whorls of stem branches, and are assuming the aspect of a tree. Taproots reach depths of 3 to 4 feet, and the strongest laterals extend 6 to 8 feet. The period between the eighth and twelfth years of the young tree's life witnesses the following developments:

(1) An acceleration in the growth rate of primary lateral branches, as a result of which the radial lateral spread of the root system becomes approximately twice the length of the taproot, and 1.5 to 3 times the length of the stem. Hence, the lateral roots displace the taproot as the most prominent feature of the root system.

(2) Retardation of the growth rate of the taproot as it penetrates the subsoil below the 3-foot level.

(3) The attainment of linear equivalence of stem and taproot, prior to which the taproot was longer, and after which the stem is always longer. The difference in length between stem and taproot constantly increases with the growth of the tree.

(4) The attainment of conspicuous size by secondary and tertiary branches on the stronger primary laterals.

Root growth between the ages of 12 and 30 years, or, on the basis of stature, between the heights of 4 and 25 feet, is characterized as follows:

(1) Continuous elongation of the primary laterals until lengths of 25 to 35 feet are attained.

(2) Maintenance of a ratio of about 1.5 to 1 between the length of the strongest laterals and the height of the tree.

(3) Maintenance of a ratio approximating 1 mm to 1 foot between basal diameters and lengths of lateral roots.

(4) Continuous elongation of branches of the higher orders, increasing the density of roots within the occupied volume of soil.

(5) Continuous elongation of vertical roots, which, however, after reaching depths of 3 to 4 feet, grow much more slowly than the surface laterals, and practically cease to grow at depths of 8 to 9 feet.

As a result of the processes listed above, a constantly increasing proportion of the root system as a whole becomes localized in the surface soil.

After trees have reached a height of about 25 feet, and a diameter breast high of about 4 inches, root development enters gradually upon a different phase. It is characterized as follows:

(1) Cessation of elongation by the stronger primary laterals and by the taproot and stronger sinkers.

(2) Marked thickening of the basal 2 or 3 feet of the stronger primary laterals. The increment usually is greater in the vertical plane, forming narrowed, planklike roots; in many cases, the increment is added almost entirely on the top side, resulting in eccentrically thickened, buttresslike supporting roots. This basal thickening increases the mechanical strength of the central root system, and in all probability is initiated by the stresses incident to an enlarging crown.

(3) Continued elongation of the smaller primary laterals until they approximate the length of the stronger ones, after which they, too, undergo basal thickening.

(4) Some continued growth of branches of the higher orders, resulting in increasing density of roots in both the topsoil and the subsoil.

(5) A tendency toward marked thickening of the innermost sinkers. Such thickening occurs in response to the requirements of the tree for support, hence the development is inversely proportional to the strength of the taproot.

Lateral roots function mechanically as props or braces, and only incidentally as guy wires. Thickening is stimulated by compression, and not by tensile strain. This is conclusively demonstrated by the eccentric development of the root systems of leaning trees.

Poor development of tops is associated with inferior root systems.

Root branches, irrespective of size, can be traced inward to the primary xylem of the parent root, thus indicating that they originated when that region of the parent root was young. Hence, a root system poorly branched in youth will always be poorly branched, and will predispose toward weakness of the tree. Adventitious root branches appear to be absent in pitch pine, except that in rare cases they may develop in wound tissue following clean severance of a root.

Replacement tips develop following the death of the terminal portion of a root only when death has not extended back into the region where secondary wood completely encircles the primary xylem.

Pitch pine is capable of extensive root growth below the water table in saturated soils. The descending branches of taproots under water sometimes are arranged in a peculiar and characteristic fan-shaped mass, the explanation of which is obscure. Trees growing on saturated soils appear to be in full health and vigor.

On sloping sites, the primary lateral branches generally parallel the soil surface, both uphill and downhill.

On heavier soils, root development tends to be less extensive, both horizontally and vertically, than it is in the sandy soils of the Coastal Plain.

Root fusions occur occasionally, on both *Pinus rigida* and *P. echinata*, between roots of the same or of different systems.

The fine roots of the higher orders, through which most absorption presumably occurs, are most profusely developed in the upper soil layers, and frequently extend into the raw surface humus. The ultimate branches of vertical roots are generally coarser and are produced in much less profusion. Nearly all root tips become dormant, and many die, during the dry periods of midsummer. Growth takes place mostly in the spring and fall.

Mycorrhizae are a conspicuous feature of pitch pine roots. Their period of growth coincides with that of nonmycorrhized tips. They are found at all depths on both drained and saturated soils, but attain their greatest profusion in the surface organic layers.

With respect to adaptations to xeric sites, the root systems of pitch pine show no apparent superiority over those of many other species of trees.

Certain suggestions are offered with respect to silvicultural practices.

The root development of *Pinus echinata* in New Jersey is fundamentally similar to that of *P. rigida*. The most marked difference is the tendency of *P. echinata* to develop a much stronger and more massive taproot, with which is correlated a weaker development of secondary supporting roots. This species does not invade areas where the water table is high.

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THE IDENTIFICATION OF CERTAIN VIRUSES AFFECTING LEGUMINOUS PLANTS[1]

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INTRODUCTION

There are numerous reports in the literature concerning the transmission of legume mosaic viruses to various hosts. The failure, however, to describe adequately the viruses and the diseases which they cause has resulted in much confusion. The overlapping in the host range of certain viruses makes it impossible in many instances to identify a virus from a brief description of symptoms or a mere report of successful transmission from one host to another. In a previous paper (*17*)[3] it was shown that several distinct viruses may infect the common bean, *Phaseolus vulgaris* L. The symptom expression on differential hosts, the host range, and the properties of the viruses concerned were found to be sufficiently definite to permit identification and specific naming. Recently, certain other legume mosaic viruses have been studied for the purpose of accumulating evidence that beans, peas, and other leguminous plants may also be subject to a number of different viruses and that these viruses may be readily differentiated and identified in various ways. Additional data on the common bean mosaic virus (bean virus 1) and the yellow bean mosaic virus (bean virus 2) previously studied and the results of a comparative study of five other viruses affecting leguminous plants are presented in this paper.

REVIEW OF LITERATURE

A mosaic disease of sweet pea, *Lathyrus odoratus* L. described by Taubenhaus (*28*) in 1914 was shown by him to be transmissible by aphids and by artificial methods. Later reports on leguminous plant viruses by McLarty (*13*), Elliott (*5*), Dickson (*3*), Doolittle and Jones (*4*), Böning (*1*), Merkel (*14*), Zaumeyer (32), Zaumeyer and Wade (*33*), Henderson (*9*), Weimer (*29*), and Johnson (*10*) also were concerned primarily with accounts of symptoms and of transmission experiments. The actual identity of the viruses concerned was in most cases not established beyond the fact that they were found causing a mosaic on some particular host or hosts and were transmissible to certain others.

There are, on the other hand, reports of legume plant viruses in which the identity of the viruses was sufficiently established to per-

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[2] The author gratefully acknowledges his indebtedness to Dr. C. W. Hungerford for helpful advice given during the course of this investigation; to Dr. Merl Stubbs for helpful cooperation in the indentification of certain viroses; to Drs. James Johnson and J. C. Walker for suggestions regarding the manuscript; to Dr. W. C. Snyder for supplying certain diseased specimens; and to Ruth Remsberg for photographs.

[3] Reference is made by number (italic) to Literature Cited, p. 1038.

mit their recognition by other workers. Thus in the studies on common bean mosaic by Reddick and Stewart (*22, 23, 24, 27*), Fajardo (*6, 7*), Nelson (*15*), Pierce and Hungerford (*18*), and Pierce (*17*) it seems apparent that the same virus (bean virus 1) was used in all cases. Kendrick and Gardner (*8, 11*) established that a seed-borne soybean mosaic virus was nontransmissible to garden beans, 7 other species of *Phaseolus*, 2 species of *Dolichos*, field peas, and cowpeas. It would appear that this virus is quite specific to soybean, and not confusable with the yellow bean mosaic virus (bean virus 2) and an alfalfa mosaic virus (alfalfa virus 2) which Pierce (*17*) found transmissible to soybean as well as to bean and a number of other leguminous hosts. The two latter viruses (bean virus 2 and alfalfa virus 2) were studied (*17*) comparatively with the virus of common bean mosaic (bean virus 1) and all were found to be distinct and separate entities.

Osborn (*16*) has described a pea mosaic which he obtained from plants of *Vicia faba* L. The virus concerned produced distinctive enations on the under surfaces of infected crimson clover and pea leaves. Stubbs [4] in an intensive study of viroses of the garden pea differentiated two distinct viruses capable of causing pea mosaic. One of these was found to produce enations similar to those described by Osborn (*16*). This virus was also found to infect the Perfection variety of peas, and was designated by Stubbs [5] as enation pea mosaic (pea virus 1). The other virus was incapable of causing infection on Perfection peas, and was named pea virus 2.

Certain well-defined viruses commonly associated with nonleguminous plants have in some instances been found to affect plants of the family Leguminosae. Thus the tobacco ring spot virus was shown by Wingard (*31*) to be transmissible to bean and sweetclover. Pierce (*17*) extended the host range to include still other legume plants. Price (*19*) found that the ordinary tobacco mosaic virus (tobacco virus 1) would produce local lesions on bean, and Carsner (*2*) showed that the virus of curly top of sugar beets was transmissible to bean by means of the beet leaf hopper *Eutettix tenellus* (Baker). Severin and Henderson (*25*) reported common beans, lima beans, cowpeas, horsebeans, vetch, hairy Peruvian alfalfa, chickpea, and a number of clovers as susceptible to the curly top virus. In studying the host range of the virus of southern celery mosaic (celery virus 1), Wellman (*30*) found that following inoculation, broadbean (*Vicia faba*) developed small purplish primary lesions which did not become systemic. Price (*20*) found that certain strains of cucumber mosaic virus produced necrotic primary lesions on cowpea, *Vigna sinensis* (L.) Endl. One strain was found to cause a typical mosaic disease of cowpea.

Linford (*12*) reported the transfer of pineapple yellow spot from *Emilia sagittata* (*Vahl*) DC. to peas by *Thrips tabaci* Lindeman. Symptoms on peas were described as streak and as similar to symptoms noted on peas naturally infected with a streak.

It is evident from these accounts that there are a number of viruses capable of infecting leguminous plants, and that the mere observation of a virus infection on any particular legume host cannot necessarily

[4] STUBBS, M. W. VIROSES OF THE GARDEN PEA (PISUM SATIVUM L.). Ph. D. thesis, University of Wisconsin. 1935. (Unpublished manuscript.)
[5] STUBBS, M. W. See footnote 4.

be considered as a new and separate disease specific to that particular species.

EXPERIMENTAL MATERIALS, METHODS, AND HOSTS

VIRUSES STUDIED

The viruses studied in this investigation were obtained from various legume plants affected with viroses. The fact that many of the legume plants used were found to be susceptible to more than one virus makes it inaccurate and confusing to speak of the virus of the mosaic disease of red clover, for instance, without regard to which specific virus may be responsible for the virosis in question. An attempt has been made, therefore, to differentiate these viruses sufficiently to name them. In order to simplify the designation of the various viruses for the reader, the names have been applied at the beginning of the paper, rather than at the close. The viruses studied were as follows:

Common bean mosaic virus (*bean virus 1*).—This virus is the one responsible for the common and prevalent type of mosaic affecting common beans (*Phaseolus vulgaris*) and which has been described by Reddick and Stewart (*22*, *23*, *24*, *27*), Fajardo (*6*, *7*), Nelson (*15*), Pierce (*17*), and others. The virus was secured from mosaic-infected bean seedlings and was used in this study in inoculation tests on beans only.

Yellow bean mosaic virus (*bean virus 2*).—This virus was previously (*17*) described and named. In this study the virus was obtained from a mosaic-infected yellow sweetclover, (*Melilotus officinalis* (L.) Lam.) plant grown in the field at Moscow, Idaho, in 1934. Symptoms on bean and thermal death point studies established this as identical with the yellow bean mosaic virus (bean virus 2) previously studied.

White clover mosaic virus (*white clover virus 1*).—This virus was secured from a white clover (*Trifolium repens* L.) plant naturally infected with mosaic at Moscow, Idaho. The same virus was obtained also from naturally infected yellow trefoil. The virus is transmissible to beans, sweetclover, red clover, and peas. It causes necrosis of many varieties of peas. Differential hosts, thermal death point, and aging experiments are described in detail later in the paper.

Enation pea mosaic virus (*pea virus 1*).—This virus was obtained from peas, *Pisum sativum* L., naturally infected with mosaic in the field. The infected specimens were grown in California. This virus produces enations on the under surfaces of leaves of affected peas similar to those described by Osborn (*16*) and Stubbs.[6] It is referred to in this paper as pea virus 1 since it is believed to be the same virus as that described by Stubbs.

Common pea mosaic virus (*pea virus 3*).—This virus was obtained from a mosaic pea seedling of the Horsford variety grown in the greenhouse of the Department of Plant Pathology, University of Idaho, Moscow, Idaho. The specificity of the virus was suspected from the fact that it was not transmissible to beans. As shown later, this virus is similar in symptom expression to a group of viruses studied by Stubbs [6] as pea viruses 2 A, B, and C; but since certain differences

[6] STUBBS, M. W. See footnote 4.

have been noted, the virus used in this study is designated as the common pea mosaic virus (pea virus 3).

Soybean mosaic virus (*soybean virus 1*).—This virus was secured from plants grown from mosaic-infected seed of the Midwest variety of soybeans (*Soja max* (L.) Piper) obtained from the Purdue Agricultural Experiment Station, LaFayette, Ind. As shown later, this virus is undoubtedly the same as the one described by Gardner and Kendrick (*8*, *11*).

A virus obtained from red clover (*Trifolium pratense* L.) and which produces local necrotic lesions on the small-seeded broadbean (*Vicia faba* var. *minor*) has been studied in a preliminary way. For purposes of designation this virus is referred to in this paper as the broadbean local-lesion virus. Final naming is reserved until such time as the host range has been studied and a more complete description worked out.

METHODS AND TEST HOSTS

The studies were made in the greenhouse where the temperature was usually held at about 25° C. Inoculum was prepared by crushing leaves and stems of infected plants in a sterile mortar and then removing gross solids by straining through cheesecloth. Inoculations were made on young plants by rubbing the leaf surfaces with a cheesecloth pad that had been immersed in the inoculum. The percentage infection was greatly increased by the use of carborundum powder, as described by Rawlins and Tompkins (*21*). In these tests, however, the carborundum powder was added directly to the inoculum just prior to making inoculations.

The thermal death point determinations were made by pipetting 2 cc of a 1 to 1 dilution of freshly extracted infective juice into thin-walled test tubes. The tubes were stoppered and placed in an agitated constant-temperature water bath for 10 minutes at the desired temperature, after which the tubes were rapidly cooled in cold running water. Inoculations were then made immediately to various test hosts. The aging tests were made by storing inocula in stoppered test tubes in a darkened cupboard at a temperature of 20° to 22° C. The virus extracts were tested at desired intervals by removing about 2 cc from the storage tubes and inoculating young test plants in the usual manner.

The test hosts used were: A French variety of dwarf edible-pod peas secured from a commercial seed company at Moscow, Idaho, under the name "Nain mangetout à longue cosse" (*Pisum sativum* var. *saccharatum* Hort.); a mosaic-free strain of Stringless Refugee Green beans (*Phaseolus vulgaris*); a strain of small-seeded broadbeans (*Vicia faba* var. *minor*); yellow sweetclover (*Melilotus officinalis*); and the Midwest variety of soybeans (*Soja max*). Each of these hosts was found to be susceptible to one or more of the viruses studied. The thermal death point and aging determinations were usually made on two or more different hosts.

EXPERIMENTAL RESULTS

The differentiation of the viruses considered in this paper was based upon symptom expression, varietal susceptibility of peas and beans, susceptibility of certain leguminous plant species, and upon certain properties of the viruses *in vitro*.

SYMPTOM EXPRESSION

The differentiation of viruses on the basis of symptoms may often lead to erroneous conclusions, but as long as the ultimate test of virus infection must be based upon symptoms of one kind or another it is essential that careful attention be given to a comparative study of symptom expression on differential hosts. In this study symptom expression has not been confined to the principal host species but is also described on other hosts that have been found to be particularly well adapted to differentiation.

Common bean mosaic virus (bean virus 1) produces the common mottled and leaf curl symptom associated with the seed-borne type of bean mosaic as shown in figure 1, *A*, and as described in detail by Fajardo (*6*), Nelson (*15*), and Pierce (*17*).

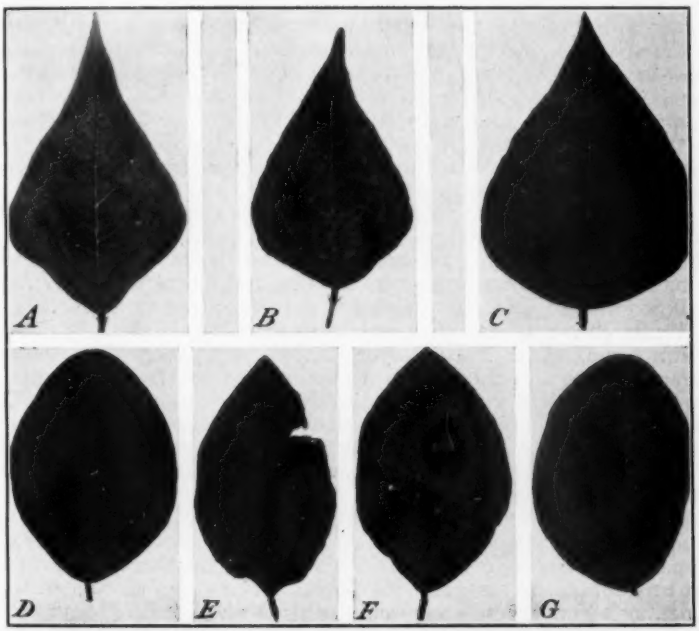

FIGURE 1.—Bean and soybean leaves showing symptoms of infection with certain viruses: *A*, Stringless Refugee Green bean infected with bean virus 1; *B*, Robust bean infected with bean virus 2; *C*, Robust bean infected with white clover virus 1; *D*, Midwest soybean, noninoculated control; *E*, Midwest soybean infected with soybean virus 1; *F*, Midwest soybean infected with pea virus 1; *G*, Midwest soybean infected with the broadbean local-lesion virus.

The symptoms caused by yellow bean mosaic virus (bean virus 2) were described fully on differential varieties of bean in a former publication (*17*); however, a few of the important symptoms of diagnostic value are repeated here. The first symptom following inoculation of the primary leaves on Stringless Refugee Green beans is a distinctive drooping of the developing trifoliate leaf. The leaflets are definitely pointed downward from the point of attachment to the petiole. Small light-yellow spots soon develop in the dark-green

background; and the yellowing gradually spreads over more or less of the surfaces of the young trifoliate leaves, producing a definite yellow mosaic pattern. Figure 1, *B*, shows typical symptoms on the Robust variety of beans. None of the other viruses tested on beans produced the distinctive downward pointing of the young trifoliate leaf.

On certain varieties of peas, bean virus 2 produces a typical mosaic pattern (fig. 2, *B*). The pea mosaic caused by this virus, however, was somewhat milder than the pea mosaics caused by pea virus 1 and pea virus 3, as shown in figure 2, *B*, *C*, and *D*.

The symptoms produced by enation pea mosaic virus (pea virus 1) on peas was usually very severe, and consisted of mottling, crinkling, and savoying of the leaves and stipules (fig. 3, *A*). On very susceptible varieties like Alderman, necrotic spots appear, accompanied by proliferations on the under surfaces of the leaves, as shown in figure 3, *B*. Since these enations were not found on any plants affected with other viruses they are believed to be of especial diagnostic value. The pea mosaic of Osborn (*16*) is undoubtedly the same as enation pea mosaic, since he found enations as a constant symptom. This virus is believed to be the same as that causing extreme malformation of pods as described by Snyder (*26*). This virus also affects soybeans, producing a mottled dark and light-green pattern as shown in figure 1, *F*.

Common pea mosaic virus (pea virus 3) causes a distinct mottling consisting of yellow and green patterns on peas. In figure 2, *C*, is shown typical mottling symptoms on Alaska peas. This virus also produces mosaic mottling on broadbean, red clover (fig. 4, *D*), and yellow sweetclover. No symptoms were produced on beans.

White clover mosaic virus (white clover virus 1) produces a very distinct mosaic on white clover, *Trifolium repens*. There is also some dwarfing and crinkling as shown in figure 5, *A*. The symptoms on red clover and yellow sweetclover are particularly severe. Distinct mottling is accompanied by crinkling of the leaves, and in some cases a slight spot necrosis is evident on red clover leaves (fig. 4, *B*). Yellow sweetclover plants are definitely dwarfed, crinkled, and mottled when infected with white clover virus 1.

Under winter greenhouse conditions a complete necrosis of peas was caused by this virus (fig. 5, *F*). This complete killing was due in part to secondary attack by damping-off fungi. Necrosis is caused on broadbeans, but usually the plants survive and show a distinct mottling together with some malformation and necrotic spots in the leaves (fig. 5, *E*). White clover virus 1 is also transmissible to beans and produces some local vein necrosis on Stringless Refugee Green, followed by a diffused yellow mottling in the subsequent developing leaves. This virus does not produce the distinctive drooping effect on the young trifoliate leaves which is characteristic of infections with bean virus 2. Figure 1, *C*, shows diffuse mottling on the Robust variety. Symptoms on alfalfa are mottling and malformation as shown in figure 5, *C*.

Soybean mosaic caused by soybean virus 1 is characterized by mottling of a more or less mild type under greenhouse conditions. The leaves are usually curled downward and malformed with dark-green areas interspersed over a light-green (chlorotic) background. Figure 1, *E*, shows typical leaf symptoms following inoculation of the

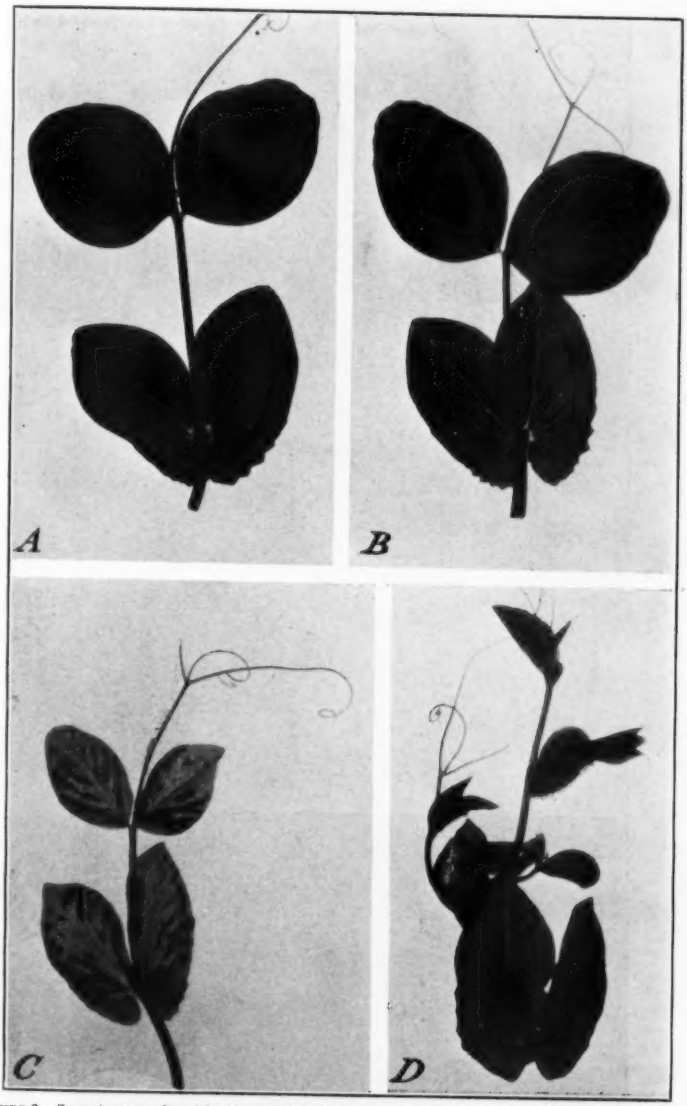

FIGURE 2.—Symptoms produced by three different viruses on Alaska peas: *A*, Noninoculated control; *B*, infected with bean virus 2; *C*, infected with pea virus 3; *D*, infected with pea virus 1. Note twisting and malformation characteristic of infections with virus used in *D*.

FIGURE 3.—Symptoms on peas produced by infection with pea virus 1: *A*, Naturally infected mosaic pea plant from which pea virus 1 was obtained; *B*, lower surfaces of leaflets showing enations produced by pea virus 1.

Midwest variety of soybean. The symptoms and effect on yield have been fully described by Gardner and Kendrick (*8, 11*).

The broadbean local-lesion virus produces distinct local necrotic lesions on the small-seeded broadbean, *Vicia faba* var. *minor* (fig. 6, *A, B*). These lesions are a dark chocolate brown in color. The virus did not become systemic on broadbean. On peas it caused a necrosis of the veinlets which was followed by a distinct chlorosis and dropping of the leaves. In figure 6, *D* and *E*, are shown typical symptoms on Progress and Horal varieties of peas. The symptoms appear first on the lower leaves and then progressively upward, in

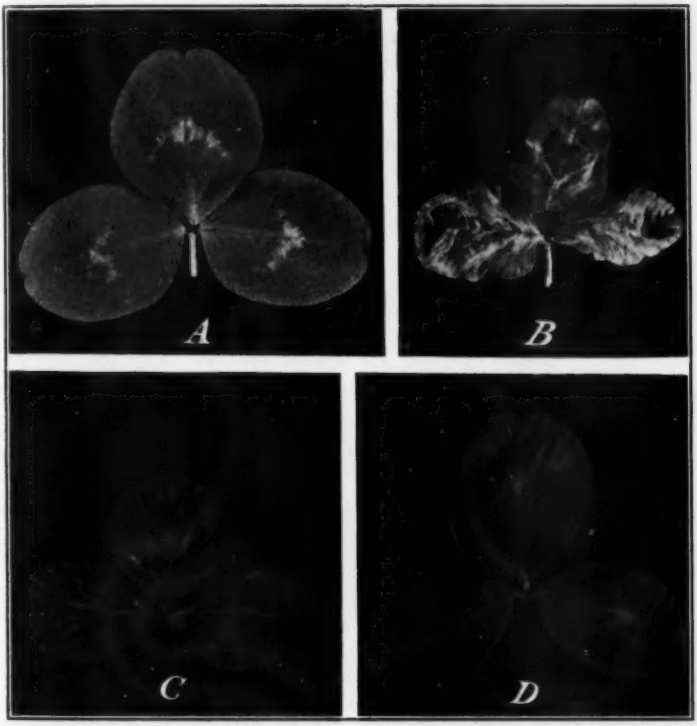

FIGURE 4.—Leaf symptoms produced by three different viruses on red clover: *A*, Noninoculated control; *B*, infected with white clover virus 1; extreme crinkling and malformation is characteristic; *C*, infected with bean virus 2; *D*, infected with pea virus 3. Note that symptoms produced by bean virus 2 and pea virus 3 are very similar.

some cases killing the plants. More often, however, the disease does not affect the upper part of the plants. On soybean this virus produces a slight chlorosis, as shown in figure 1, *G*.

RESISTANCE AND SUSCEPTIBILITY IN PEA VARIETIES

Each of the viruses studied was tested on the following varieties of garden peas, *Pisum sativum:* Alaska, Perfection, Wisconsin Early Sweet, Green Admiral, Surprise, Progress, Alderman, Dwarf Tele-

phone, and Horal. These tests were made more for the purpose of establishing differences in the viruses than in showing differences in varietal susceptibility. However, it will be seen that certain very

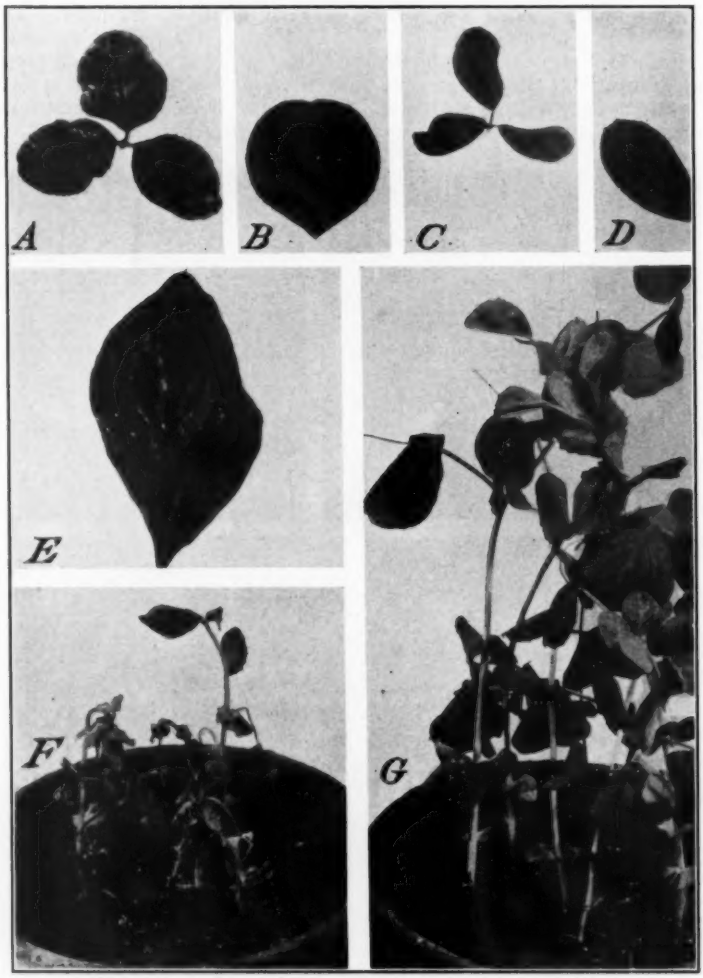

FIGURE 5.—Symptoms on various hosts produced by white clover virus 1: *A*, Infected white clover; *B*, noninoculated white clover; *C*, infected common alfalfa; *D*, noninoculated alfalfa; *E*, infected small-seeded broadbean; *F*, infected Nain mangetout à longue cosse variety of peas showing complete necrosis; *G*, noninoculated peas.

definite differences in varietal susceptibility were established, which may be of value to plant breeders seeking to develop resistant varieties.

Inoculations were made in the regular routine manner; carborundum powder was added directly to the inoculum and the leaf surfaces were then rubbed with a cheesecloth pad which had been immersed in the inoculum. No attempts were made to reinoculate plants; thus in certain instances in which varieties were not 100 percent infected, it cannot necessarily be concluded that the remainder were entirely resistant, but rather that they merely escaped infection. In table 1 the number of plants inoculated of each variety is given together with the percentage infection obtained with the various viruses.

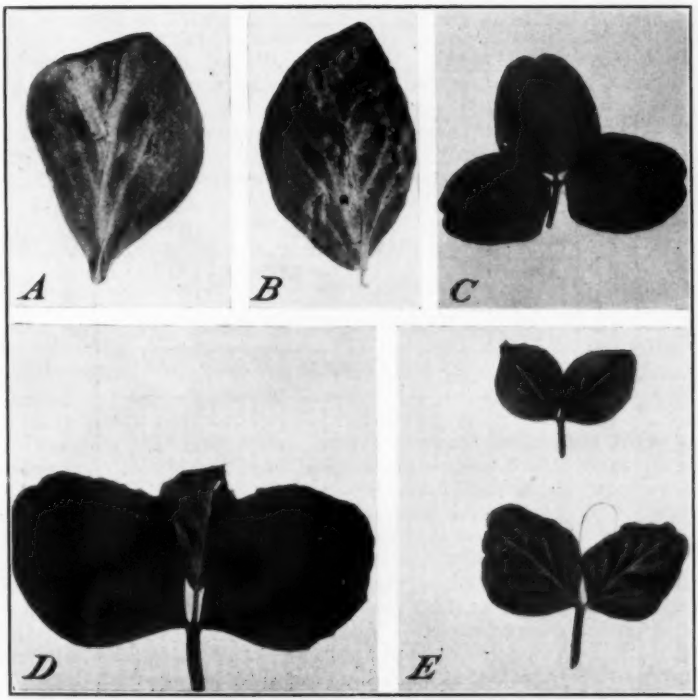

FIGURE 6.—Symptoms on various hosts produced by the broadbean local-lesion virus: *A*, broadbean control, inoculated with water; *B*, local lesions on broadbean; *C*, red clover infected with both the broadbean local-lesion virus and bean virus 2; *D*, Progress pea leaves infected with the broadbean local lesion virus; *E*, Horal pea leaves infected with broadbean local-lesion virus, note necrosis of veinlets.

In a previous paper (*17*) it was reported that the variety Perfection was resistant to infection with bean virus 2. The same was found to be true in the present investigation; however, certain other varieties of peas have been found to be susceptible. Alaska, Green Admiral, Alderman, and Dwarf Telephone were all found to be very susceptible (table 1). No infection was secured on Perfection and Horal varieties.

Pea virus 1 was transmitted to all of the varieties of peas tested (table 1). The varieties Perfection and Horal, which were found

TABLE 1.—*Relative susceptibility of certain plant species and of certain horticultural varieties of peas and beans to white clover virus 1, bean virus 1, bean virus 2, pea virus 1, pea virus 3, soybean virus 1, and the broadbean local-lesion virus as determined by artificial inoculations on greenhouse-grown plants*

Species	Common name or horticultural variety	White clover virus 1		Bean virus 1		Bean virus 2		Pea virus 1		Pea virus 3		Soybean virus 1		Broadbean local-lesion virus	
		Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
		Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Pisum sativum	Alaska	55	96	------	------	77	73	20	30	71	94	15	0	20	35
	Alderman	15	100	------	------	28	71	14	57	24	100	9	0	15	33
	Dwarf Telephone	21	95	------	------	37	65	12	75	26	92	14	0	14	36
	Green Admiral	40	100	------	------	25	86	29	59	36	92	15	0	------	------
	Horal	26	73	------	------	44	0	15	60	34	0	13	0	17	53
	Perfection	62	92	------	------	83	0	14	36	60	5	14	0	20	10
	Progress	20	100	------	------	22	32	20	55	20	60	15	0	15	67
	Surprise	30	97	------	------	36	36	22	41	31	77	10	0	19	47
	Wisconsin Early Sweet	37	100	------	------	20	10	22	50	24	25	13	0	20	15
P. sativum var. *saccharatum*	Nain mangetout à longue cosse	28	100	5	0	20	80	20	75	39	84	10	0	21	62
	Stringless Refugee Green	24	92	26	100	41	93	10	0	28	0	22	0	25	0
Phaseolus vulgaris	Common Great Northern	22	23	24	75	25	72	24	0	17	0	15	0	------	------
	Great Northern UI No. 1	15	73	22	0	25	68	25	0	22	0	20	0	------	------
	Robust	19	26	23	0	33	82	23	0	25	0	20	0	------	------
	Idaho Refugee	15	40	80	0	20	80	17	0	15	0	15	0	------	------
	Wisconsin Refugee	22	27	94	0	23	74	17	0	15	0	16	0	------	------
Vicia faba var. *minor*	Broadbean	17	88	------	------	59	69	15	73	14	86	18	0	------	------
Melilotus officinalis	Yellow sweetclover	40	100	------	------	55	84	15	0	24	67	15	0	------	------
Soja max	Midwest soybean	15	0	------	------	22	9	20	85	15	0	60	85	10	80
Trifolium pratense	Red clover	35	54	------	------	40	35	10	0	20	25	10	0	------	------
Trifolium repens	White clover	35	100	------	------	10	0	10	0	10	0	10	0	------	------
Medicago sativa	Common alfalfa	20	50	------	------	10	0	10	0	10	0	10	0	------	------
Nicotiana tabacum	Connecticut Havana No. 38 tobacco	5	0	------	------	25	0	5	0	15	0	10	0	------	------
Petunia hybrida	Single mixed petunia	10	0	------	------	10	0	10	0	10	0	10	0	------	------

resistant to pea virus 3, were susceptible to infection with pea virus 1. On varieties susceptible to both viruses, however, lower percentages of infection were secured with pea virus 1 than with pea virus 3.

The pea varieties tested for susceptibility to pea virus 3 were in general more susceptible to it than to bean virus 2; however, the most susceptible varieties to bean virus 2 were also the most susceptible to pea virus 3. Alaska, Alderman, Dwarf Telephone, and Green Admiral were readily infected (table 1). No infection was obtained on Horal and only three plants of Perfection were infected. These three plants may well have been admixtures or rogues.

White clover virus 1 produced necrosis of all the pea varieties tested. On the basis of percentage of plants infected, Horal and Perfection exhibited the greatest resistance. One hunded percent infection was obtained on many varieties (table 1). Infection with this virus appeared to predispose plants to attack by damping-off fungi.

No infection was obtained with soybean virus 1 upon the varieties of peas (*Pisum sativum*) tested. Kendrick and Gardner (*11*) were unable to obtain infection on field peas in their trials.

The broadbean local-lesion virus was successfully transmitted to all the varieties of peas tested (table 1). Only a low percentage of infection was obtained on Perfection and Wisconsin Early Sweet. In these limited tests Progress was the most readily infected.

RESISTANCE AND SUSCEPTIBILITY IN BEAN VARIETIES

Six varieties of beans, *Phaseolus vulgaris*, were tested for susceptibility to the various viruses. The varieties tested were: Stringless Refugee Green, Idaho Refugee, Wisconsin Refugee, Robust, Common Great Northern, and Great Northern UI No. 1. The data are given in table 1.

Stringless Refugee Green and Common Great Northern were susceptible to the common bean mosaic virus (bean virus 1). No infection with this virus was obtained on Robust, Great Northern UI No. 1, Idaho Refugee, and Wisconsin Refugee.

All varieties were more or less susceptible to the yellow bean mosaic virus (bean virus 2). The varieties Robust, Great Northern UI No. 1, Idaho Refugee, and Wisconsin Refugee which were resistant to common bean mosaic were found to be less easily infected with bean virus 2 than was Stringless Refugee Green. These result are in accord with previous findings (*17*).

All of the bean varieties tested were susceptible to infection with white clover mosaic virus (white clover virus 1). Higher percentages of infection were obtained on Stringless Refugee Green than on any of the other varieties. The symptoms produced by this virus were considerably less severe than those produced by bean virus 2.

Enation pea mosaic virus (pea virus 1) was not transmissible to any of the bean varieties tested.

No infection on bean varieties was obtained with the common pea mosaic virus (pea virus 3). Inoculum from inoculated beans when transferred back to peas gave no infection, showing that the virus was not present and that the beans were immune from infection with this virus.

Inoculations to the six varieties of beans with the common soybean mosaic virus (soybean virus 1) failed to produce symptoms. Transfer

inoculations from inoculated beans back to soybean gave no infection, indicating that the virus was not present in beans.

Only the Stringless Refugee Green variety of beans was tested for susceptibility to the broadbean local-lesion virus. In no case was infection obtained on this variety.

RESISTANCE AND SUSCEPTIBILITY OF CERTAIN PLANT SPECIES

The following plant species were tested by artificial inoculation for susceptibility and resistance to each of five viruses: Small-seeded broadbean, *Vicia faba* var. *minor*; yellow sweetclover *Melilotus officinalis;* red clover, *Trifolium pratense*; white clover, *T. repens;* common alfalfa *Medicago sativa* L.; Midwest variety of soybean, *Soja max*; Stringless Refugee Green bean, *Phaseolus vulgaris*; Nain mangetout à longue cosse variety of peas, *Pisum sativum* var. *saccharatum*; Connecticut Havana No. 38 tobacco, *Nicotiana tabacum* L.; and garden petunia, *Petunia hybrida* Vilm.

TABLE 2.—*Summarized scheme for the differentiation of certain viruses affecting legume plants, based on data secured on greenhouse determinations of the resistance and susceptibility of certain plant species* [1]

Virus	*Phaseolus vulgaris*		*Pisum sativum*		*Melilotus officinalis*, yellow sweet-clover	*Soja max*, Midwest soybean
	Stringless Refugee Green	Robust	Alaska	Perfection		
Bean virus 1	+++	−	−	−	−	−
Bean virus 2	+++	++	++	−	++	+
Pea virus 1	−	−	++	++	−	++
Pea virus 3	−	−	+++	−	++	−
White clover virus 1	+++	+	+++	+++	+++	−
Soybean virus 1	−	−	−	−	−	+++
Broadbean local-lesion virus	−	−	++	++	(2)	+++

[1] +++ designates severe infection, ++ moderate infection, + slight infection, and − designates no infection.
[2] No data.

It was the purpose of these tests to establish, if possible, differences in the host range of each virus. Obviously the limited number of species tested cannot be considered as adequate host-range studies; however, it will be seen that the differential susceptibilities of the various species to the different viruses is of value in establishing virus identities (tables 1 and 2). Thus the pea mosaic viruses, pea virus 1 and pea virus 3, are seen to differ from most of the other viruses in being nontransmissible to bean. Pea virus 3 differs from pea virus 1 in being nontransmissible to the Perfection and Horal varieties of peas. Furthermore, in these tests pea virus 3 was not successfully transmitted to soybean, while pea virus 1 was transmitted readily. The broadbean local-lesion virus produced on broadbean only local necrotic lesions, thus differentiating it from those viruses causing systemic infection on broadbean. The host range of the broadbean local-lesion virus has not been studied. Of the five viruses used in tests with tobacco and petunia, none was transmitted, thus establishing that they were different from the many viruses known to affect tobacco and petunia of the family Solanaceae. Many other important differences in the susceptibility of the various species may be observed

from the results given in table 1. As a further aid in the identification of legume plant viruses, a summarized scheme for their differentiation on the basis of host susceptibility and resistance is given in table 2. As shown in this table, many of the viruses may be readily identified by making artificial inoculations to a few varieties of peas and beans and to yellow sweetclover and soybean. In a previous paper (*17*) it was reported that red clover was not susceptible to bean virus 2, but in the present investigation in which carborundum powder was used as an abrasive, a low percentage of infection was obtained on red clover. Furthermore, a number of red clover plants brought in from the field were found to be naturally infected with bean virus 2. It is to be expected that as more adequate methods of artificial inoculation are developed certain other species listed as resistant to certain viruses will be found to be susceptible. It is probable also that the natural insect vectors may extend the list of susceptible host plants.

PROPERTIES OF THE VIRUSES

The thermal inactivation points and resistance to aging in vitro were determined for all of the viruses studied in the hope of finding differences sufficient for a basis of separation and identification. The determinations on each virus were made in most cases on two or more different hosts. Thus the virus of common pea mosaic (pea virus 3) was tested on peas and on broadbean. Wherever possible the inoculum was secured from the same host species as that upon which the determinations were made. The results are tabulated in tables 3, 4, and 5.

Thermal Inactivation Point

(Tables 3 and 4)

The thermal inactivation point of bean virus 2 was found to lie between 58° and 60° C. when heated for 10 minutes. It was previously reported (*17*) at 56° to 58°, but in the present investigation an occasional infection was obtained with virus extracts heated at 58°.

Pea virus 1 was usually inactivated at 56° C., but in two trials infection was obtained after heating at this temperature. No infections were obtained in any trial after heating at 58° for 10 minutes.

Pea virus 3 was inactivated at 62° to 64° C. for 10 minutes in the determinations made on both peas and broadbeans.

White clover virus 1 was found not to survive the 58° C. heatings in tests on sweetclover and peas. In one test on beans this virus gave a low percentage of infection following heating at 58°.

The thermal death point determinations on the broadbean local-lesion virus were based on the ability of the virus to produce local lesions on broadbean. This virus produced few lesions following heating at 60° C. and no lesions after heating at 62° (table 4).

Soybean virus 1 was inactivated by heating at 58° C. in all tests on the Midwest variety of soybean.

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TABLE 3.—*Comparison of the thermal inactivation points of white clover virus 1, pea virus 1, pea virus 3, bean virus 2, and soybean virus 1 as determined by systemic infection on various hosts*

Temperature (° C.)	White clover virus 1 on—								Pea virus 1 on—		Pea virus 3 on—				Bean virus 2 on—		Soybean virus 1 on—	
	Peas		Broad-bean		Yellow sweet-clover		Beans		Peas		Peas		Broad-bean		Beans		Soy-bean	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
Inoculated control	8	8	8	8	10	10	16	16	30	23	16	14	15	12	21	19	45	38
50					5	5	5	5			6	5	15	8	5	4	33	25
54			8	7					25	8					5	5	35	19
56	8	8	8	3	5	5	16	8	25	2					18	13	22	15
58	8	0	8	0	10	0	16	1	30	0	10	1	15	6	20	2	22	0
60	8	0	8	0	10	0	16	0	32	0	16	1	15	4	16	0	30	0
62									26	0	10	1	15	1				
64											10	0	10	0				

TABLE 4.—*The thermal inactivation point and resistance to aging in vitro of the broadbean local-lesion virus as determined by the production of local lesions on Vicia faba var. minor* [1]

Temperature (° C.)	Test 1	Test 2	Test 3	Test 4	Time aged	Test 1	Test 2
	Number	*Number*	*Number*	*Number*	*Hours*	*Number*	*Number*
Inoculated control	480	660	213	208	0	480	213
50		610			24	146	120
56					30	30	
58	6		10		48	20	5
60	0	6	1	9	72	0	0
62	0		0	0			
65		0					

[1] Numbers are total lesions produced on 20 inoculated leaves of *Vicia faba* var. *minor*.

TABLE 5.—*Comparative tests on resistance to aging in vitro of white clover virus 1, pea virus 1, pea virus 3, bean virus 2, and soybean virus 1, as determined by systemic infection on various hosts*

Time aged (days)	White clover virus 1 on—						Pea virus 1 on peas		Pea virus 3 on—				Bean virus 2 on—				Soy-bean virus 1 on soy-bean	
	Peas		Yellow sweet-clover		Beans				Peas		Broad-beans		Beans		Peas			
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	No.	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*	*No.*
0	6	6	5	5	5	5	30	23	15	12	13	11	5	5	7	5	25	25
1							20	14	5	4	7	3	5	5	8	6	34	28
2	8	8	5	5	5	5	20	1	16	8	21	7	5	0	5	0	32	12
3	12	12	5	5	5	5	20	0	16	0	23	0	5	0	5	0	30	0
5	8	8	5	5	7	6												
7	8	0	7	0	5	0												

The difference in the thermal inactivation points of the various viruses, while in most cases small, were in some instances sufficient to be of value in comparative differentiation.

Resistance to Aging in Vitro

(Tables 4 and 5)

Bean virus 2 was inactivated after aging 24 to 48 hours, which was in accord with previous findings (*17*).

Pea virus 1 was found to be noninfectious after aging 3 days.

Pea virus 3 was still infectious after aging 48 hours but failed to infect either peas or broadbeans after 72 hours.

White clover virus 1 withstood agings of 5 days, but after this length of time symptoms were slower in developing than in the control tests, indicating that probably the concentration of infective virus had been considerably reduced. No infection was obtained after aging 7 days.

The broadbean local-lesion virus and soybean virus 1 were both inactivated by aging 3 days.

The differences in the ability of certain of the viruses to withstand aging in vitro are sufficient in most cases to show definitely that separate and distinct viruses were concerned.

Experiments on Virus Separation and Combination

A red clover plant taken from the field in 1934 was found to be infected with two separate and distinct viruses. One of these was the broadbean local-lesion virus (fig. 6, *B*) and the other has been identified as bean virus 2 (fig. 4, *C*). These conclusions were arrived at in the following manner: The original virus infection on red clover produced mottling accompanied by some necrosis as shown in figure 6, *C*. When the infected red clover was used as a source of inoculum and inoculations were made directly to the Nain mangetout à longue cosse variety of peas, a necrosis and streaking developed as shown in figure 7, *A*. When inoculations were made direct from red clover to the small-seeded broadbean, local necrotic lesions developed within 4 or 5 days; and 4 to 6 days later systemic symptoms appeared in the new growth. Inoculations to peas with the systemic mosaic material from broadbean as inoculum caused only a mild mottling in peas, as shown in figure 7, *B*. It seemed probable, therefore, that on broadbeans the local lesions were caused by one virus and the systemic infection by another, and that the combination of the two viruses on peas produced necrosis. The systemic entity on broadbean was studied alone, and in tests on Stringless Refugee Green beans appeared to be identical with bean virus 2. The original combination from red clover produced only systemic infection typical of that produced by bean virus 2 in comparative inoculations to bean. Furthermore, inoculations from bean back to broadbean produced only systemic infections, indicating that beans were not susceptible to the local-lesion entity.

The isolation of the local-lesion entity from the combination on red clover was accomplished by making inoculations to the Horal or Perfection varieties of peas which are resistant to bean virus 2 but susceptible to the local-lesion virus. Figure 6, *E*, shows Horal peas infected with the local-lesion virus alone.

DISCUSSION

The purpose of the work described in this paper has been primarily to differentiate certain viruses that affect leguminous plants. It is hoped ultimately to work out a more complete description of each virus which will include host range, properties, transmissibility through seed, insect vectors, and occurrence in nature on economic hosts. In the meantime it appeared essential to develop short methods of identification, so that the virus causing a particular mosaic disease, could be readily identified by making artificial inoculations to a few differential hosts or by property tests. The necessity for such a method of identification can readily be appreciated from the frequent use in the literature of such terms, for instance, as the virus of red clover mosaic, without recognition of the fact that there is or might be anyone of several viruses capable of causing a mosaic

FIGURE 7.—Symptoms produced on Nain mangetout à longue cosse peas: *A*, By combination of bean virus 2 and the broadbean local-lesion virus; *B*, by bean virus 2 alone.

symptom on red clover. It is realized that the identification of certain viruses will, in many instances, be a difficult task, especially in cases where two or more viruses are present in the same plant. On the whole, however, it is believed that more careful attention on the part of workers dealing with legume mosaics to the identification of the particular viruses concerned will go far toward clearing up the confusion existing in this particular field of virus study.

A few of the important facts pertaining to various viruses known to affect peas and beans have been brought together in table 6, not only to show that these hosts may be subject to several different viruses, but also to aid in the identification of the viruses. Data on certain viruses reported by other workers are included. Under the heading, "Differential hosts and diagnostic characteristics", at least one

TABLE 6.—*A partial list of viruses capable of infecting beans and peas, with notations on certain characteristics and properties useful in their differentiation and identification*

Host and virus	Host symptom	Thermal death point	Longevity in vitro	Differential hosts and diagnostic characteristics	Authority
		° C.	*Days*		
Common bean, *Phaseolus vulgaris:*					
Bean virus 1	Leaf curl and mottling	56–58	1	Seed-transmitted in bean. Robust, Great Northern UI No. 1, Idaho Refugee, and Wisconsin Refugee, immune.	Fajardo (*6, 7*), Nelson (*15*), Pierce (*17*).
Bean virus 2	Yellow mosaic	58–60	1 to 2	Not seed-transmitted in bean. Foregoing bean varieties susceptible. Perfection peas resistant.	Pierce (*17*), this paper.
White clover virus 1	Slight local necrosis and mild mosaic	58	6 to 7	Necrosis of peas. Severe mosaic on sweetclover, red clover, and white clover.	This paper.
Alfalfa virus 2	Local necrotic lesions	62–64	7 to 9	Local lesions on many varieties of bean, including Red Valentine.	(*17*). Zaumeyer and Wade (*33*).
Tobacco virus 1	Small local lesions	90	(1)	Systemic on tobacco. Local lesions on *Nicotiana glutinosa* and certain varieties of bean. Red Valentine immune.	Price (*19*), Pierec (*17*).
Tobacco ring spot virus	Local and systemic necrosis	66	7 to 9	Ring spot symptom on tobacco	Wingard (*31*), Pierce (*17*).
Garden pea, *Pisum sativum:*					
Pea virus 1	Mottling, distortion, and enations	58	2 to 3	Not transmissible to bean. Perfection and Horal peas and Midwest soybean susceptible.	Osborn (*16*), Stubbs,[2] this paper.
Pea virus 2A, B, C	A, marble; B, speckle; C, mild		½ to 1	Not transmissible to bean, soybean, red clover, or Perfection peas.	Stubbs.[2]
Pea virus 3	Yellow mosaic mottling	62–64	2 to 3	Not transmissible to beans, soybeans, or Horal peas. Transmissible to red clover. Rarely seed-transmitted.	This paper.
White clover virus 1	Necrosis	58	6 to 7	(See under bean)	Do.
Broadbean local-lesion virus	Veinlet necrosis, streak	60–62	2 to 3	Local lesions on small-seeded broadbean. Not transmissible to beans.	Do.
Alfalfa virus 2	Top necrosis on Perfection	62–64	7 to 9	Local lesions on bean. Necrosis on Perfection peas.	Pierce (*17*).
Tobacco ring spot virus	Top necrosis	66–70		Ring spot symptom on tobacco	Stubbs.[2]
Pineapple yellow spot virus	Streak			Transmitted by *Thrips tabaci*	Linford (*12*).
Bean virus 2	Mild mosaic	58–60	1 to 2	(See under bean)	This paper.

[1] 3 months or more.
[2] STUBBS, M. W. See footnote 4.

characteristic is given wherein a specific virus differed from the other viruses affecting the same host. Certain differences in the thermal death points and resistance to aging in vitro also are of value in identifying some of the viruses. It should be pointed out, however, that the thermal death points of most of the legume mosaic viruses studied were grouped rather closely around 60° C. This fact may be of significance in the classification of viruses; that is, if the thermal death points of certain groups of viruses are found to be correlated to some extent with the type of host plant affected, a more or less natural grouping may eventually be made. With a view to eventual classification, it would appear advantageous to consider the legume mosaic viruses, the cane-fruit mosaic viruses, the solanaceous mosaic viruses, and so forth, each as separate groups; and to separate and differentiate the viruses and the virus strains within each group by extensive and intensive comparative studies as to plant species and horticultural varieties susceptible, transmissibility by insects and by artificial methods, transmissibility through seed, and physical properties.

The transmissibility of the various viruses through seed was not studied. However, a large number of seedlings of various varieties of peas were grown for inoculation purposes, and it seems significant that only a single case of seed transmission was observed throughout the course of the study. The virus obtained in this one case of seed transmission was studied and named common pea mosaic virus (pea virus 3).

It may be well to consider here the possible identity of certain viruses referred to in the literature. The pea mosaic described by Doolittle and Jones (*4*) and the broadbean mosaic of Böning (*1*) would seem on the basis of host relationship to have been caused by the same virus as the one described in this paper as pea virus 3. This may also have been the same virus that Zaumeyer and Wade (*33*) noted on red clover and that they found to be nontransmissible to bean. Stubbs[7] was unsuccessful in transmitting his pea virus 2 *A*, *B*, *C* to red clover, and therefore considered his strains as distinct from those of Doolittle and Jones (*4*), Böning (*1*), and Zaumeyer and Wade (*33*). It must be admitted, however, that the symptom expression of Stubbs' pea virus 2*A* is strikingly similar to that of pea virus 3. There can be little question but that the pea mosaic virus of Osborn (*16*) was the same as the virus later described by Stubbs and referred to in this paper as pea virus 1.

The viruses affecting certain legume hosts which Zaumeyer and Wade (*33*) found to be transmissible to beans may have been either bean virus 2, white clover virus 1, or both. The alfalfa mosaic virus of Zaumeyer and Wade (*33*) which produced only necrotic local lesions on bean was probably the same virus as that described by the writer in a previous paper (*17*) as alfalfa virus 2. The alfalfa mosaic virus which Weimer (*29*) found to be nontransmissible by artificial means should in all probability be considered as a separate and distinct virus. Henderson's (*9*) new virosis of sweetclover which he found to be transmissible to tobacco and petunia but which differed from the tobacco ring spot virus, must have been caused by a virus distinct from any studied in this investigation since none of the latter was found to be transmissible to tobacco.

[7] STUBBS, M. W. See footnote 4.

The broadbean local-lesion virus described in this paper which tends to produce streak symptoms on peas, especially when in combination with other viruses, may or may not be the same as Linford's (*12*) streak. A further study of the host range of this virus would undoubtedly give significant evidence one way or the other.

It seems to be sufficiently evident from these investigations that many leguminous species are susceptible to several different viruses. Further progress in and understanding of the viroses of legumes is dependent, therefore, upon recognition of this fact, and upon comprehensive studies of the viruses themselves as well as of the diseases caused by them.

SUMMARY

The investigations reported in this paper were concerned primarily with the differentiation and identification of seven viruses affecting leguminous plants. The viruses studied were: The common bean mosaic virus (bean virus 1); the yellow bean mosaic virus (bean virus 2); a white clover mosaic virus (white clover virus 1); enation pea mosaic virus (pea virus 1); common pea mosaic virus (pea virus 3); the common soybean mosaic virus (soybean virus 1); and a virus obtained from red clover which was designated as the broadbean local-lesion virus. Differentiation was based upon symptom expression on differential hosts, varietal susceptibility of peas and beans, susceptibility of certain leguminous plant species, and upon certain properties of the viruses in vitro.

For practical purposes of identification the most significant differences between viruses was found in their host ranges and in varietal susceptibility of peas and beans. Pea viruses 1 and 3 were not transmissible to beans by the artificial inoculation methods used. Differentiation of pea virus 1 from pea virus 3 was made on the basis of resistance and susceptibility of pea varieties and upon differences in host range. Pea virus 1 infected soybean while pea virus 3 did not.

Bean virus 1 and bean virus 2 were readily differentiated on the basis of susceptibility and resistance of bean varieties. Bean virus 2 was transmissible to certain varieties of peas.

White clover virus 1 was differentiated from the other viruses studied on the basis of its ability to infect all varieties of beans and peas tested, and on the basis of symptom expression.

The broadbean local-lesion virus differed from all the other viruses studied in its ability to produce local necrotic lesions at the point of inoculation on the small-seeded broadbean.

Soybean virus 1 appeared to be specific to soybean, no infection on other species being obtained.

The differences in host ranges of the various viruses were supported in part by differences in symptom expression, differences in longevity in vitro, and in some cases by slight differences in the thermal death points of the viruses.

The differences established were believed to be sufficient to allow for the recognition of these viruses by other investigators.

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THE VITAMIN A, B, C, D, AND G CONTENT OF THE OUTER GREEN LEAVES AND THE INNER BLEACHED LEAVES OF ICEBERG LETTUCE [1]

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INTRODUCTION

The association of vitamin A with greenness of plants has been a subject of much interest almost since the discovery of this vitamin. In 1925, studies were begun in the Bureau of Home Economics to determine the vitamin A content of the outer green leaves and the inner bleached leaves of Iceberg lettuce, a variety of *Lactuca sativa*. While the results of this study were being prepared for publication the report of Dye, Medlock, and Crist (3) [2] on the association of vitamin A with greenness in plant tissues appeared. Publication was therefore delayed, and the studies were extended to include vitamins B, C, D, and G. The present report covers all of these studies, although some of the evidence obtained merely corroborates the findings reported by other investigators.

The tests were completed before the International vitamin standards of reference became available for general use. Inasmuch as the purpose of the study was to determine the relative vitamin potency of the green and the bleached leaves, the results are still valid even though they cannot be interpreted in terms of International units.

MATERIAL

All tests were made with Iceberg lettuce purchased on the Washington retail market. Most of it was grown in California. The inner bleached leaves were from the head as it is offered for sale by the retailer, care being taken to discard all leaves showing an appreciable amount of greenness. The outer green leaves were those generally trimmed from the head before it is sold. These were secured regularly by a special arrangement with the grocer. As soon as the lettuce was received it was carefully washed and stored in a covered receptacle in the refrigerator until it was to be used. The excess water was patted off with a dry towel before the portions for the rats were weighed. The thick part of the stem at the base of the leaves was discarded.

PROCEDURE AND RESULTS

VITAMIN A

The vitamin A tests were made by the Sherman-Munsell technique (7). The basal diet was irradiated to supply vitamin D. The green leaves were fed at levels of 0.0125, 0.025, 0.05, 0.1, 0.2, and 0.8 g per

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[2] Reference is made by number (italic) to Literature Cited, p. 1046.

rat 6 times per week, and the bleached leaves at levels of 0.4, 0.8, and 1.2 g. The results, given in table 1, indicate that the green leaves contained about 34.5 Sherman units per gram while the bleached leaves contained only 1 unit per gram. Kramer and her associates (*5*) in 1929 reported results indicating that the green leaves were 30 or more times as rich as the bleached leaves.

TABLE 1.—*Vitamin A content of Iceberg lettuce*

Leaves fed	Quantity fed per rat 6 times per week	Rats used in test	Average gain in weight of rats during 8 weeks	Standard error of mean gain of rats	Estimated quantity of lettuce to give 25 g gain in 8 weeks	Vitamin A units (Sherman) per gram of lettuce
	Grams	*Number*	*Grams*	*Grams*	*Grams*	
Outer green	0.0125	7	[1] 8.7	2.7	0.029	34.5
	.025	11	22.1	3.7		
	.05	6	39.5	5.6		
	.1	7	55.3	9.0		
	.2	6	73.2	8.6		
	.8	6	66.0	12.6		
Inner bleached	.4	7	5.2	7.7	1.0	1.0
	.8	14	22.7	3.6		
	1.2	13	27.9	3.3		

[1] Average of 6 surviving animals.

VITAMIN B

Tests for vitamin B were made according to the method described by Chase and Sherman (*2*). Rats, weaned at the age of 4 weeks, were given the vitamin-B-free diet. At the end of 2 weeks they were placed in individual cages and allotted portions of lettuce as their only source of vitamin B (B_1).

A short time before these tests were begun a new colony of rats had been established in the laboratory from a few animals obtained from the Wistar Institute. This colony was maintained on the same stock diet as the regular colony established some 10 years ago from which animals are drawn for vitamin B and vitamin G tests. One assay was made with young rats from this new colony (B) in order to check their behavior when under test. The other assay was made with animals from the regular stock colony (A). The results of both assays are shown in table 2.

The green leaves were fed at levels of 2, 4, and 6 g and the bleached leaves at levels of 1, 2, 4, and 6 g in the test with rats from colony A. In the second series with rats from colony B, both the green and bleached leaves were fed at levels of 2, 4, and 6 g. With one exception where only one female was used, each group of animals on the different levels of lettuce contained the same number of males and females. Furthermore, for the most part each individual of a group on any one level of a given kind of lettuce was paired with a litter mate of the same sex in the groups on the other levels. These precautions are essential to the derivation of Sherman units, and also make more valid the comparison between the results obtained with the green and bleached leaves.

TABLE 2.—*Vitamin B content of Iceberg lettuce*

Description of leaves fed	Tests with rats from colony A						Tests with rats from colony B					
	Quantity fed per rat 6 times per week	Rats used in test [1]	Average gain (+) or loss (−) in weight of rats during 8 weeks	Standard error of mean gain of rats	Vitamin B units (Sherman) per gram of lettuce	Ratio of vitamin B content of green to bleached leaves	Quantity fed per rat 6 times per week	Rats used in test [1]	Average gain (+) or loss (−) in weight of rats during 8 weeks	Standard error of mean gain of rats	Vitamin B units (Sherman) per gram of lettuce	Ratio of vitamin B content of green to bleached leaves
	Grams	*Number*	*Grams*				*Grams*	*Number*	*Grams*			
Outer green	2	5 M, 5 F	−11.1	3.8	0.24	0.80	2	8 M, 8 F	−2.4	2.8	0.27	0.69
	4	5 M, 5 F	+21.5	2.4			4	8 M, 8 F	+30.8	1.8		
	6	2 M, 2 F	+50.5	------			6	8 M, 8 F	+56.4	3.3		
Inner bleached	1	5 M, 5 F	[2] −11.9	2.6	.30		2	8 M, 8 F	+14.4	3.1	.39	
	2	5 M, 5 F	+2.8	3.2			4	8 M, 8 F	+52.6	3.4		
	4	5 M, 5 F	+36.1	3.0			6	8 M, 8 F	+75.1	5.0		
	6	1 F	+51.0	------								

[1] M=male and F=female. [2] Average of 8 surviving animals.

In the test with animals from colony A the ratio of potency of green leaves to bleached was 0.80, while in the second test it was 0.69. This is a rather close agreement considering the variability of results obtained by the biological method. In 1931 Kohman, Eddy, and Gurin (*4*) reported findings indicating that the outer green leaves were appreciably richer in the vitamin B complex than the inner bleached leaves.

VITAMIN C

Tests for vitamin C content, conducted according to the technique of Sherman, LaMer, and Campbell (*6*), were made at two different times. In the early tests the levels of lettuce fed to the guinea pigs were 6, 12, 15, 18, and 21 g per day. The results indicated that 21 g might be near the minimum protective dose, but the limited data obtained did not warrant forming definite conclusions. The assays have, therefore, been repeated recently with daily feedings of 15, 18, and 21 g. The results of the tests at these levels are given in table 3. All animals survived the full 90 days of the test period. The minimum protective dose of the green leaves seems to be slightly more than 21 g and of the bleached leaves about 21 g. Kohman, Eddy, and Gurin (*4*) have reported the minimum protective dose as between 15 and 25 g. They state that there is very little difference between the green and the bleached leaves.

TABLE 3.—*Vitamin C content of Iceberg lettuce*

Quantity of lettuce fed per guinea pig 6 times per week (grams)	Tests with outer green leaves				Tests with inner bleached leaves			
	Guinea pig no.[1]	Total gain in weight of guinea pig during 90 days	Degree of scurvy	Scurvy score	Guinea pig no.[1]	Total gain in weight of guinea pig during 90 days	Degree of scurvy	Scurvy score
		Grams				*Grams*		
15	B23 M	220	Trace	3.5	B26 M	348	None	0
	B31 M	260	Mild	5.5	B33 M	226	Moderate	11.5
	42 M	25	do	8	B34 M	171	do	3.5
	53 M	72	Moderate	2	43 M	196	Trace	2.5
	356 M	366	Trace	3	355 F	120	do	3
	377 F	151	Moderate	7	366 M	145	do	2
	383 M	366	Trace	2	375 M	204	Mild	5
					382 F	179	None	0
	Average	208.6		4.4		198.6		3.4
18	55 M	−23	Moderate	13	57 F	191	Mild	1.5
	56 M	118	Mild	2	83 M	264		7
	59 M	135	do	1.5				
	80 M	108	Trace	3				
	84 M	369		2				
	357 F	253	Trace	2	354 M	363	Trace	2
	365 M	20	Mild	7	373 F	288	do	1
	378 F	279	Trace	4	380 F	211	do	1
	379 M	345	do	1				
	Average	178.2		3.9		263.4		2.5
21	82 M	349	Moderate	0.5	81 F	267		2.5
	86 M	316		0	85 M	246		0
	364 F	53	Moderate	9	89 F	268		0
	371 M	222	Trace	3	368 F	155	None	0
					372 M	341	Trace	2
	Average	235		3.1		255.4		.9

[1] M = male and F = female.

VITAMIN D

The vitamin D tests were made by the usual line-test technique. Rats 28 days old were placed on a Steenbock yellow-corn low-phosphorus ration until they developed severe rickets. They were then given nine daily feedings of lettuce. At the end of the ninth day they were killed and the line test was made. The lettuce was fed at levels of 2, 3, and 4 g and one rat received 5 g. No healing was produced even at the 5-g level. This would seem to be sufficient evidence that lettuce does not contain detectable amounts of vitamin D.

VITAMIN G

The vitamin G tests were made according to the technique worked out in this laboratory. It is practically the same as the method described by Bourquin and Sherman (*1*), except that an alcoholic extract of rice polishings is used as a source of vitamin B. In these tests as in the vitamin B tests, two assays were made, one with animals from colony A and the other with animals from colony B. In the first test the outer green leaves were fed at levels of 0.5, 1, and 2 g and the inner bleached leaves at levels of 1, 2, and 4 g. In the tests with animals from colony B both kinds of leaves were fed at levels of 2, 4, and 6 g. As in the tests for vitamin B, care was taken

to have equal numbers of males and females in each group of animals on the different levels of lettuce, and as far as possible to have the groups fed one kind of lettuce contain a representative of the same sex from each litter of test animals used.

In these vitamin G assays very different results (table 4) were obtained with the rats from the two colonies. In the first test made during the late summer and fall the green leaves assayed 0.46 Sherman unit per gram and the bleached leaves 0.24 unit per gram. In the second test made in spring and late summer the green leaves gave a value of 1.18 units and the bleached leaves 0.67 unit per gram. The ratios between green and bleached for the two tests were respectively 1.91 and 1.76. Thus the relative potency shown by the results of the two tests was very similar although the absolute values were very different. More recent work in this laboratory has corroborated this variable behavior of rats from different colonies when they are confined on a vitamin-G-deficient diet. Of course there is a possibility that the differences may have been due to seasonal variation in the vitamin content of the lettuce although there are no data to substantiate this.

TABLE 4.—*Vitamin G content of Iceberg lettuce*

Description of leaves fed	Tests with rats from colony A, late summer and fall						Tests with rats from colony B, spring and late summer					
	Quantity fed per rat 6 times per week	Rats used in test [1]	Average gain in weight of rats during 8 weeks	Standard error of mean gain of rats	Vitamin G units (Sherman) per gram of lettuce	Ratio of vitamin G content of green to bleached leaves	Quantity fed per rat 6 times per week	Rats used in test [1]	Average gain in weight of rats during 8 weeks	Standard error of mean gain of rats	Vitamin G units (Sherman) per gram of lettuce	Ratio of vitamin G content of green to bleached leaves
	Grams	*Number*	*Grams*				*Grams*	*Number*	*Grams*			
Outer green	0.5	5 M, 5 F	8.4	3.2	0.46	1.91	2	8 M, 8 F	44.1	3	1.18	1.76
	1	5 M, 5 F	18.5	6.1			4	8 M, 8 F	62.9	2.1		
	2	5 M, 5 F	23.7	2.9			6	8 M, 8 F	80.4	3.7		
Inner bleached	1	5 M, 5 F	13.6	3.2	.24		2	8 M, 8 F	29.9	3	.67	
	2	5 M, 5 F	19.4	5.4			4	8 M, 8 F	40.7	3.1		
	4	5 M, 5 F	24.4	3.9			6	8 M, 8 F	47.9	3.4		

[1] M=male and F=female.

SUMMARY

The outer green leaves and the inner bleached leaves of Iceberg lettuce (*Lactuca sativa*) were assayed for vitamins A, B, C, D, and G.

The vitamin A content was derived as 34.5 units (Sherman) per gram in the green and 1 in the bleached leaves.

Two assays for vitamin B made with test animals from two different colonies showed values of 0.24 and 0.27 unit (Sherman) per gram for the green and 0.30 and 0.39 for the bleached leaves.

The vitamin C test indicated that the minimum protective level for the green leaves was slightly more than 21 g. Very nearly complete protection was obtained with 21 g of the bleached leaves.

Vitamin D could not be detected in either the green or the bleached leaves, although quantities as high as 5 g per day were fed to the test animals.

Vitamin G assays, likewise made with animals from two colonies, showed in the one case 0.46 unit (Sherman) per gram for green leaves and 0.24 for the bleached. In the other test the values were 1.18 and 0.67 respectively. The ratios of potency of green to bleached leaves in the two tests, however, were 1.91 and 1.76.

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